CHEMISTRY OF NATURAL PRODUCTS

SUMMARY

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
CHEMISTRY
TO
THE ALIGARH MUSLIM UNIVERSITY
ALIGARH.

BY
SATYA PRAKASH

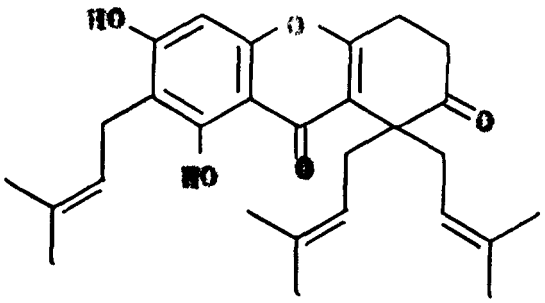
DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH.
1981

SUMMARY

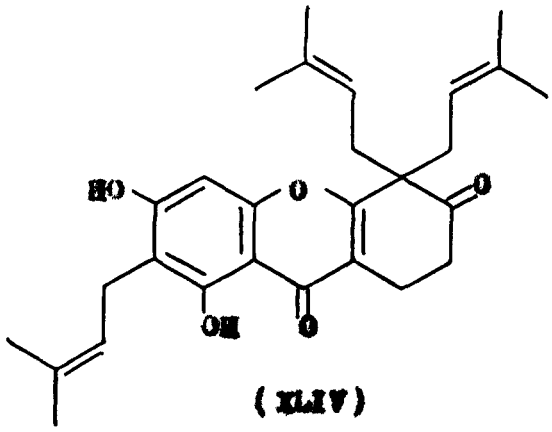
Investigations of a number of medicinal plants were carried out and results of some of these which merit chemical interest are reported in the thesis. The major part of the thesis is devoted to analysis of the structure of a xanthone which was isolated from Calophyllum wightianum. Xanthones are not as wide spread in the plant kingdom as flavonoids and coumarins and spectroscopic information of value in their structural elucidation is still not so readily available as in the case of flavonoids and coumarins. The same is true of xanthone biogenesis. The recent literature on xanthones is, therefore, reviewed in some detail in the theoretical section of the thesis.

The structural work on the xanthone from Calophyllum wightianum (N.O. Guttiferae) was complicated by the presence of a fatty acid impurity in almost stoicheiometric proportions. In fact the product isolated was a xanthone-fatty acid clathrate. There was, therefore, a lack of correspondence between the NMR and mass spectra and the elementary analysis did not fit any reasonable structure. The fatty acid impurity was ultimately got rid of through crystallisations from a large excess of urea which carried away the contaminating fatty acid and almost pure

xanthone was thus obtained. Analysis of the spectra of the purified xanthone solved the structural problem to the extent that it was shown to be either (XLII) or (XLIV) but differentiation between these two structures was not possible. In the mean time Sultanbawa and his co-workers reported a xanthone from Calophyllum gaylanicum to which they allotted structure (XLII). A close scrutiny of their paper, however, shows that they have not given due consideration to the alternative structure (XLIV) and it was still necessary to find convincing evidence in favour of either of the two structures.



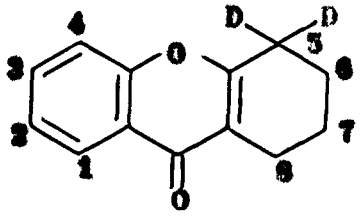
(XLII)



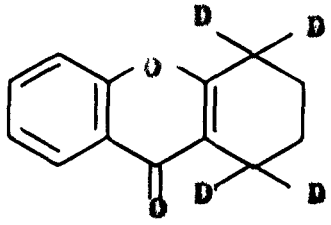
(XLIV)

It appeared that the problem could be sorted out through deuterium exchange under basic conditions if deuterium incorporation occurs only at the site indicated in (XLVII). To check this the unsubstituted tetrahydroxanthone was prepared and exposed to D_2O in basic medium. The NMR spectrum, however, showed that deuterium had entered not only at C-5 but also C-8

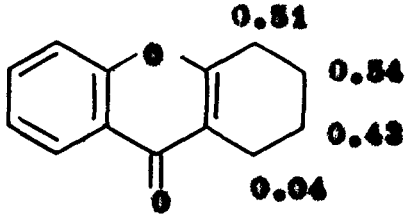
(XVIII) and, therefore, this approach could not be applied. The problem was ultimately resolved by computing the chemical shifts of the hydrogens attached to C-5, C-6 and C-7, C-8 on the basis of the chemical shifts of the corresponding hydrogens in tetrahydroxanthone (XLIV) and cyclohexanone (L) in benzene. Application of this method leaves no doubt that the correct structure is (XLIV) and so different from the one assumed by Sultambawa et al.



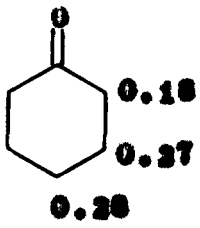
(XLVII)



(XLVIII)



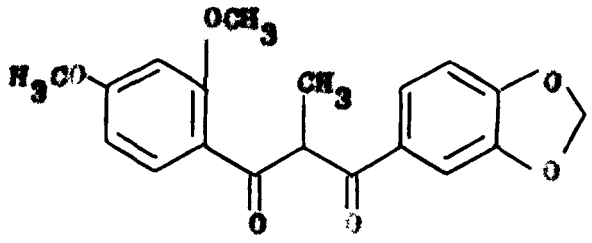
(XLIX)



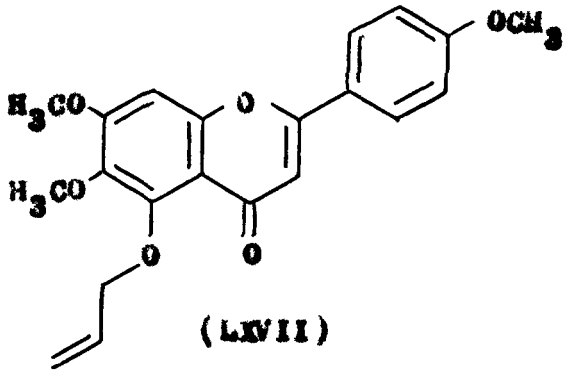
(L)

From Tinospora malabarica (N.O. Menispermaceae) the novel dibenzoyl ethane (LVII) was isolated and its structure established through analysis of its spectra and comparison with a

sample obtained through methylation of the corresponding dibenzoyl methane. The same plant yielded a flavone carrying an allyl instead of the usual γ,γ-dimethylallyl side chain. It was shown to have structure (LXVII).

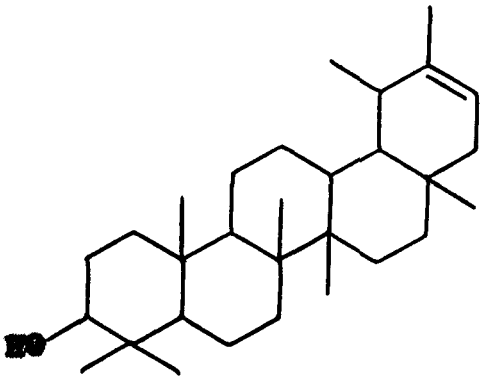


(LVII)

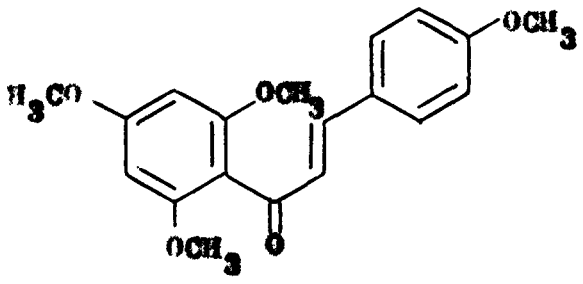


(LXVII)

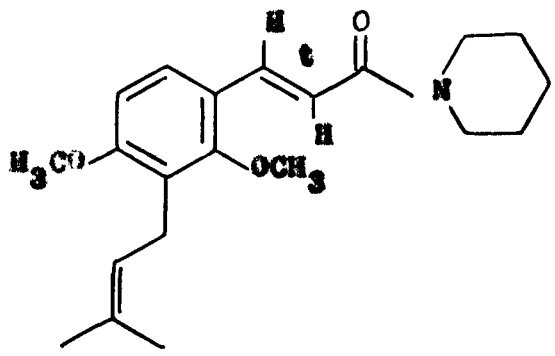
From Verbesina encaloides (N.O. Compositae) the triterpene ψ-taraxasterol and its acetyl derivative were obtained in rather large amounts and it was, therefore, considered worthwhile to work out appropriate conditions for carrying out some useful reactions employed in terpene chemistry. This was done to some extent also because in the initial stages presence of an impurity caused some confusion in its identification and it seemed likely that the compound had an additional double bond.



And lastly the extract of *Excoecaria agallocha* (N.O. Euphorbiaceae) afforded a chalcone (LXXVI) and an oil which positive Dragendorff test showed to be alkaloidal in nature. Its structure (LXXIX) follows from the NMR spectrum coupled with benzene induced shifts and is in accord with biogenetic requirements.



(LXXVI)



(LXXIX)

CHEMISTRY OF NATURAL PRODUCTS

THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

CHEMISTRY

TO

THE ALIGARH MUSLIM UNIVERSITY

ALIGARH.

BY

SATYA PRAKASH

DEPARTMENT OF CHEMISTRY

ALIGARH MUSLIM UNIVERSITY

ALIGARH.

1981

This work described in the thesis was carried out by the candidate, Mr. Satya Prakash, personally. It has not been submitted for any other degree, either of this or any other University.

(Dr. Asif Zaman)
Supervisor

ACKNOWLEDGEMENTS

I acknowledge with deep gratitude the noble inspiration I received during my tenure as a research scholar from my mentor and guide, Dr. Asif Zaman, Ph.D. (Erlangen, West Germany), Reader, Department of Chemistry, A.M.U., which introduced me into the fascinating realms of scientific investigations. I am indeed very much indebted to him for invaluable guidance, constant encouragement and advice in structuring this thesis and for his characteristic willingness to give so freely of his time.

I express my deep sense of gratitude to Dr. Francis M. Dean, The Robert Robinson Laboratories, Liverpool, U.K., for his sagacious and invaluable suggestions and for some 220 MHz NMR spectra. I am also thankful to Drs. Abu Shoeb and D.S. Bhakuni, C.D.R.I., Lucknow, who generously made some spectral data available.

I am grateful to Prof. W. Rahman, Head, Department of Chemistry, A.M.U., for providing necessary facilities. For extending every possible help at all stages I also sincerely thank Dr. K.M. Shamsuddin and Dr. Hafizullah Khan.

It gives me pleasure to insert a word of thanks to Mr. Surendra Prasad Nademi for his help in general checking of the manuscript. Thanks are also due to my colleagues for their cooperation and useful discussion.

Finally, I am also grateful to the Council of Scientific and Industrial Research, New Delhi, for financial assistance.

(SATYA PRAKASH)

C O N T E N T S

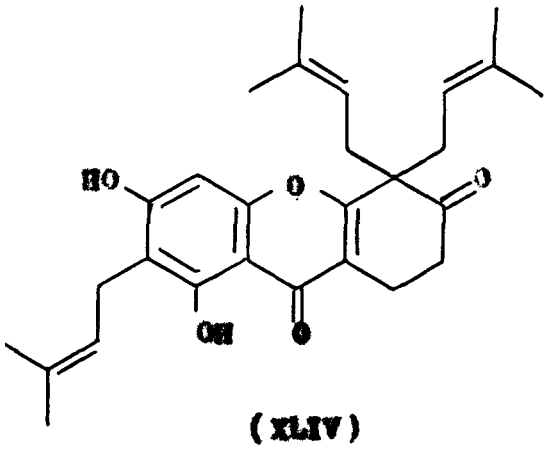
INTRODUCTION

INTRODUCTION

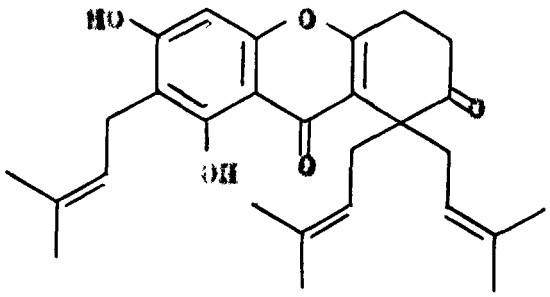
The work described in this thesis was carried out in connection with the isolation and characterisation of the secondary metabolites of indigenous medicinal plants. The drugs taken up during the course of this work were selected mostly from those generally used in the Unani system of medicine and the object in each case was the isolation, identification and, in the case of new compounds, structural elucidation. Some other plants not generally used in the Unani system of medicine were selected because of the reported isolation of either pharmacologically active or chemically novel compounds from related species.

Toxic and medicinal properties are ascribed to various species of the genera Calophyllum. The most intensively investigated species is C. inophyllum which possesses antirheumatic activity and several medicinal uses are claimed for it. Calophyllum wightianum attracted attention because as a sister species it might possess similar medicinal properties. Also xanthones, which are invariably found in Calophyllum species, present interesting structural features and xanthone glycosides have acquired a position as pharmacodynamic compounds. The largest part of the thesis is devoted to the tetrahydroxanthone,

wightianone (XLIV), elucidation of the structure of which was complicated by the presence of an impurity in almost stoichiometric amounts. While this work was in progress Sultanbawa et al reported the same compound from Calophyllum paviaticum but their structural conclusions differ (XLII). Since xanthones are a very important class of phenolic natural products which have been much in the news recently the literature on xanthones has also been reviewed to bring out the interesting aspects of their chemistry and biogenesis.



(XLIV)

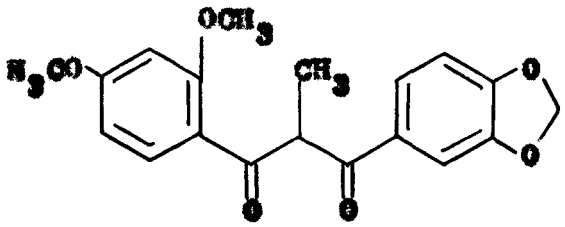


(XLII)

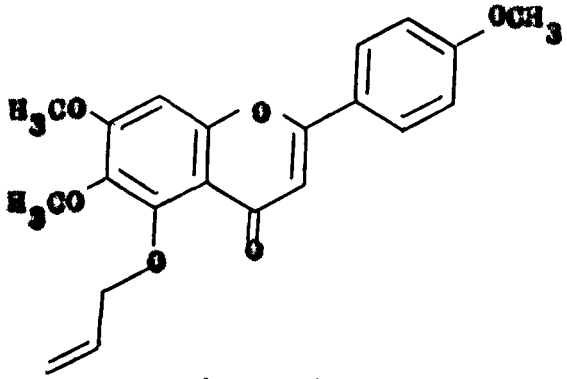
The investigation of Calophyllum wightianum demonstrates some interesting chemotaxonomic aspects regarding the unique nature of the indigenous varieties of Calophyllum. It has been suggested that the presence of jacareubin and/or its immediate precursor could be considered as a chemotaxonomic marker for this genus since in all species, excluding the Indian variety of Calophyllum inophyllum, these metabolites are present. Our

results on C. wightianum also point in the same direction since neither jasorubin nor its benzophenone precursor could be detected even in traces.

Tinospora malabarica is a Unani drug extensively used as an alterative, antipyretic, diuretic, febrifuge, stomachic etc. and is an antirheumatic drug in China. Investigation of the plant led to the isolation of two new compounds, tinosporinone (LVII) and 5-allyloxy, 4',6,7-trimethoxyflavone (LXVII) besides the bitter principle, gilloin, earlier isolated from Tinospora cordifolia. This constitutes the first isolation of a 1,1-dibenzoyl ethane and a flavone having an allyloxy grouping from a natural source.



(LVII)

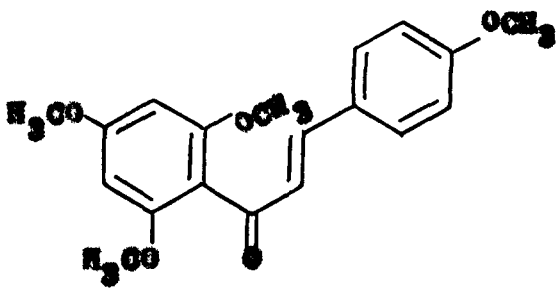


(LXVII)

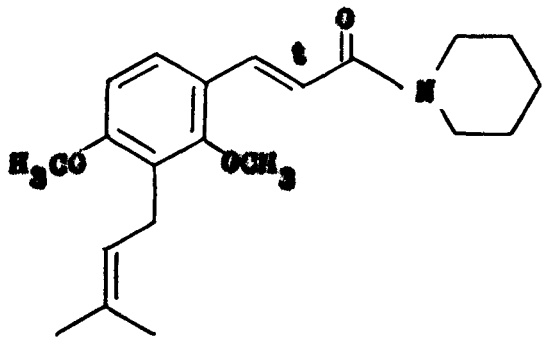
Verbesina encelioides, though not a Unani drug, was investigated because of the reported isolation of eudesmane type sesquiterpenes from sister species. The two crystalline

compounds isolated from it were identified as ψ-taraxasterol and its acetyl derivative. ψ-Taraxasterol is a member of comparatively rare group of triterpenes. Since these compounds, specially the acetate, were isolated in large amounts some reactions of value in triterpene chemistry were also studied.

Excoecharia agallocha is used as a purgative, alterative and in epilepsy though not much credence can be placed on such reports. Chemical examination of the drug revealed the presence of two products, a chalcone (LXXVI) and a new piperidine alkaloid (LXXIX). It is interesting to note that such piperidine alkaloids have so far been reported only from Piper species. This, as far as could be ascertained, is the first time this type of alkaloid has been found to occur in other families. The presence of a prenyl side chain attached to the aromatic ring also makes it the first member of this group of alkaloids having a γ,γ-dimethylallyl substituent.



(LXXVI)



(LXXIX)

THEORETICAL

XANTHONES

INTRODUCTION

The number of naturally occurring xanthones upto 1961 was limited to eighteen.[1,2] In the eight years following about four to five times this number came to light and the 'Handbook of naturally occurring compounds by F.K. Devon and A.I. Scott[3] published in 1975 records about 150 xanthones including dimeric members. The same handbook, however, lists 317 flavonoids (excluding flavans) and 96 isoflavonoids. The reason for the numerical inferiority of xanthones is that they are not as wide spread in the plant kingdom as other phenolics being confined, by and large, to three families; Guttiferae, Moraceae and Gentianaceae. Xanthones eluded early detection also perhaps due to the absence of diagnostic colour reactions of the type employed in flavonoids. The structural work on xanthones has not always been easy and the structure of morellin was ultimately established through X-ray crystallography. In the majority of cases routine methods of structural elucidation are sufficient, NMR spectroscopy being most helpful in settling the substitution pattern. The biogenesis of xanthones has by now been studied in considerable detail specially in the case of fungal xanthones

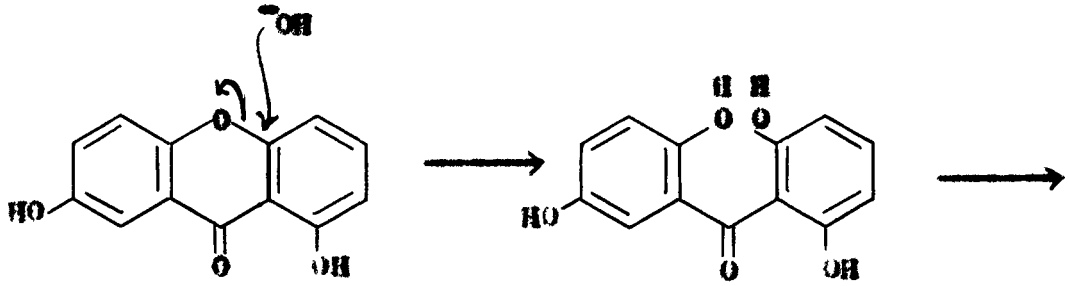
where feeding experiments are relatively easily carried out. In the following review all these aspects of xanthone chemistry are discussed in some detail.

METHODS OF STRUCTURAL ELUCIDATION

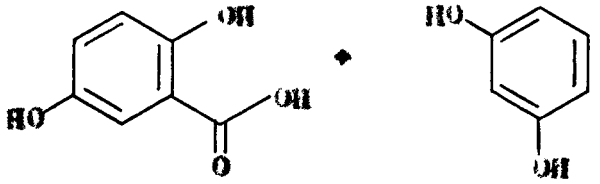
Chemically xanthones can be compared to either chromones or to their 2 and 3-phenyl substituted derivatives i.e. flavones and isoflavones respectively. Thus the carbonyl group of xanthones is inert in typical carbonyl reactions and, except for some reactivity towards Grignard reagent, does not undergo nucleophilic additions. It is also worth noting that the Mg/HCl reduction of xanthones to coloured compounds is of doubtful diagnostic importance since it may lead to products other than the coloured xanthylidine salts. The carbonyl frequency in the IR has about the same value as in flavones and intra-molecular hydrogen bonding with an adjacent hydroxyl lowers it to 1650 cm^{-1}. The UV absorption lies close to the visible region of the electromagnetic radiation and xanthones are usually cream coloured to yellow.

The problem of structural elucidation of xanthones, like that of flavonoids, centres around the assignment of hydroxyl and/or methoxyl functions and prenyl side chains to different positions of the benzene rings. In the more complex xanthones one or more heterocyclic ring may be present and its orientation has then to be determined.

The classical methods of structural elucidation rely on alkali cleavage and oxidative degradation. The course of the reaction is exemplified by the formation of gentisic acid and resorcinol from euxanthone[4](I) on fusion with potash. As in flavonoids the position most susceptible to $\bar{O}H$ attack is the one conjugated with the carbonyl group and results in cleavage of the ether linkage. The intermediate (II) can undergo further fission in two possible ways as indicated, the products isolated as already stated are gentisic acid and resorcinol. This is due to the greater reactivity (β-diketone character) of resorcinol as evident from the ready decarboxylation of α-resorcilic acid under alkaline conditions. As elsewhere in alkali fusion one has to reckon here also with concomitant demethylation of some methoxyl groups. The oxidative degradation of xanthones involving presulphate oxidation followed by destruction of the quinol ring with hydrogen peroxide is now seldom employed and the structural problem is usually settled through application of spectroscopic techniques. Where necessary further confirmation is then obtained through synthesis.

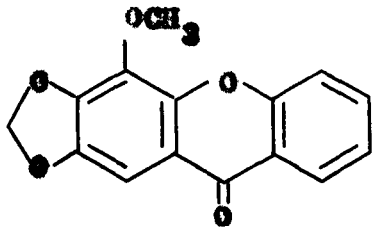


(I) (II)

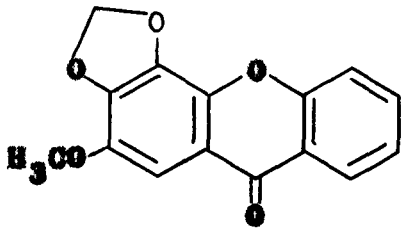


While degradative methods referred to above have lost their importance in structural elucidation it can not be claimed that NMR and mass spectrometry are sufficient to characterise unambiguously even those xanthones the structures of which are not complicated by the presence of other features. As an illustration one might usefully consider the determination of the structure of 4-methoxy, 2,3-methylenedioxy xanthone (III) obtained from Koilmeyera coriacea[5] along with other compounds having the same oxygenation pattern. The presence of methoxyl and methylenedioxy groups is evident from resonances of the methyl

and methylene protons at 5.87* and 3.76 respectively. A 1-H singlet at 2.59 and an octet at 1.62 testify to positions 2,3 and 4 being substituted and the other benzene ring having no substituents. The carbonyl group of xanthones like that of chromones and flavonoids exercises a strong deshielding influence on the benzenoid proton/protons peri to it. In the case under discussion the singlet of the lone proton appears at much higher field than the octet of the corresponding proton in the other ring as it is exposed to the shielding effect of three oxygen substituents in this ring. All these features can, however, be equally well accommodated in the alternate structure (IV) and there is no spectroscopic method to hand which can distinguish with certainty between these two alternatives. The decision in favour of (III) was reached on the basis of the following transformations.



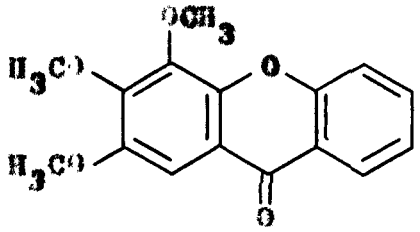
(III)



(IV)

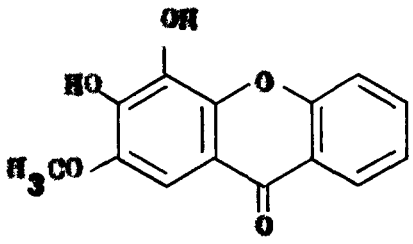
* Chemical shifts are expressed in τ throughout the thesis.

Complete dealkylation of (III) afforded a trihydroxy compound which was converted into the trimethyl ether (V). This trimethyl ether was found to undergo selective demethylation of two methoxyls in such a way that the resulting compound (VI) contained vicinal hydroxyls. When this dihydroxy compound was exposed to methylene iodide under basic conditions, it supplied a methylenedioxy methoxy compound which was not identical with



(V)

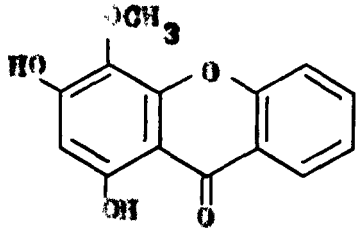
(III). Since the oxygenation pattern could not have been altered in the above reactions the two methylenedioxy methoxy compounds must be position isomers. The partial demethylation reaction was interpreted in terms of the earlier findings on demethylation of pyrogallol trimethyl ether with sulphuric acid which always led to cleavage of the central ether function. The validity of this result in the xanthone series was unequivocally demonstrated by Scheinmann et al[6,7] in connection with the structural work on jacareubin (IX). The dihydroxy compound has, therefore, to be formulated as shown in(VI) and hence the isomeric methylenedioxy compound isolated from the natural source must be assigned structure (III).



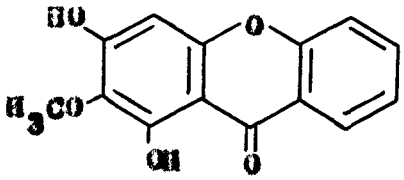
(VI)

As in flavones, coumarins etc. substituents are assigned to specific positions on the basis of chemical shifts and multiplicities of protons at adjacent positions. The influence of different oxygen functions present in the benzene ring on the chemical shifts of protons at various unsubstituted positions was studied by Ballantine et al.[8] While their calculations were initially helpful, sufficient data is now available in the literature on xanthones to allow one to assign the chemical shifts of various protons. Since only eight aromatic protons are present in the two benzene rings of an unsubstituted xanthone as against ten aromatic protons in flavone and isoflavone the NMR spectra of substituted xanthones are somewhat easier to interpret. Problems arise with xanthones having phloroglucinol type substitution when protons at 5 and 7 positions are difficult to distinguish. Thus one can not readily differentiate between (VII) and (VIII) on the basis of NMR spectroscopy and it was this type of uncertainty which made it difficult to decide whether

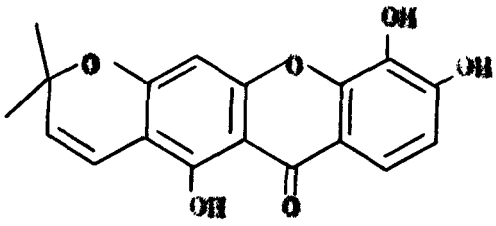
jmearubin (IX) has linear or angular structure. The problem of the structure of this compound consequently stretched out over several years and was ultimately resolved through synthesis.[9]



(VII)



(VIII)



(IX)

In the initial stages of structural work on flavones and isoflavones much reliance was put on relative acidity of the hydroxyl groups in assigning them to various positions. Thus the 7 and 4' hydroxyls in flavones are ionised by potassium or sodium bicarbonate and can therefore be methylated under milder conditions than necessary for hydroxyls at other positions. The bathochromic shifts in the UV spectra observed on introduction

of sodium acetate also afford evidence in this regard. In all polyphenols ferric colour is most useful in establishing the positions of the hydroxyl groups relative to each other and a carbonyl group, if present, and much use of this test was made in determining the substitution of flavonoids and coumarins. The presence of 5-hydroxy in flavones was routinely established through the bathochromic shift in the UV spectrum on addition of $AlCl_3$. Specially interesting as regards xanthones is the recent report[10] that the test is not given by compounds when the hydroxyl has a C-prenyl side chain adjacent to it. The Gibbs test[11,12] and the results of persulphate oxidation and Duff reaction were also important in this context. Compared to flavonoids, these methods have not been as extensively used in structure elucidation of xanthones because by the time a sufficient number of these compounds had been isolated, NMR and mass spectrometry had developed sufficiently to allow orientation problems to be settled through their use.

As discussed later in connection with their biogenesis, the oxygenation pattern of xanthones can, to some extent, be inferred from the origin of two aromatic rings through a combination of acetate and shikimate pathways so that while one ring has phloroglucinol type oxygenation the other has hydroxyls meta and para to the carbonyl. As is well known, enzymic oxidation-reduction of aromatic rings is a common occurrence and many flavones, isoflavones and xanthones exhibit pyrogallol type of

oxygenation but from a survey of the literature it seems that the gossypetin[13] type of nucleus is rather more common in xanthones than flavones.

The first step in the structure elucidation of a new natural compound is its assignment to one of the major classes into which natural products have been divided.In other words one has to decide whether one is dealing with unsaturated compounds such as flavonoids, lignans and many alkaloids or saturated polycyclic compounds such as terpenes and steroids. A cursory look at the UV spectrum of the compound is sufficient to distinguish between these two extremes. It is not as easy, however, to distinguish between closely related structural types such as flavones, isoflavones, coumarins, benzoquinones, furocoumarins and lignans from their UV spectrum. Chemists have tried to formulate generalisations of the type that were found so useful in terpenes and steroids but in the cases of the above aromatic compounds one is dealing with far more complex chromophoric systems than the dienes and enones encountered in the steroid field. Literature now a days, however, records the UV absorption data of a large number of compounds belonging to each class and the characteristic features of the UV spectra of naturally occurring aromatic heterocycles have been sought to be precisely defined.[14-17] The exercise has been successful to the extent that one can readily distinguish between, say a typical flavone and a furocoumarin but the absorption of many natural products

does not confirm to the pattern evident in the typical compounds of each class. Thus, for instance, absorption bands at 247, 274, 315 and 342 nm may be ascribed to a furocoumarin, a flavone or a xanthone. Though these values in fact belong to a flavone glycoside,[18] and vary somewhat from typical coumarin spectra, to the chemist faced with the problem of deciding the nucleus of a new natural product the minor variations in the UV spectrum are not sufficiently reliable. Consultation of the IR spectrum of the compound in combination with its UV absorption very often leads to the correct conclusion. Thus the carbonyl absorption, provided it is not distorted by intra-molecular hydrogen bonding and electron donation from other oxygen functions in the molecule, is very characteristic and distinguishes readily between α and γ-pyrone derivatives, i.e. flavonoids and coumarins.

Turning to xanthones the adduced table 1 gives the UV absorption of some di, tri and tetraoxygenated members of this class. A striking feature that emerges from a comparison of UV maxima of trioxygenated xanthones is the considerable bathochromic shift observed in the case of 1,3,5-trioxygenated compared to 2,3,4 xanthones. The magnitude of this shift is sufficient to differentiate with some certainty between 1,3,5 and 2,3,4-trioxygenated xanthones.

TABLE 1

U.V. absorption maxima, mu ( ) of xanthones

(Solvent: a = methanol, b = ethanol)

(* - shoulder, + - inflexion)

(Dioxygenated Xanthones)

Xanthone	Absorption maxima					Solvent	Ref.
1,2-dihydroxy	244 (18,900)		261 (19,400)	290* (3,000)	385 (3,800)	b	19
1,3-dihydroxy	236 (20,400)		254 (13,700)	303 (8,900)	349 (4,400)	b	19
1,4-dihydroxy	229 (22,100)		263 (24,300)	299 (6,700)	390 (3,500)	b	19
1,5-dihydroxy	237*(18,900)	247 (30,400)		316 (4,600)	379 (1,800)	b	19
1,6-dihydroxy	230 (19,900)	247 (12,300)	264 (6,600)	304 (7,300)	365 (3,700)	b	19
1,7-dihydroxy	235 (38,500)		260 (40,300)	253 (7,500)			19
1,8-dihydroxy	230 (22,800)	250 (35,100)	280 (2,500)	330 (11,400)	390 (5,400)	b	19
2,3-dihydroxy	235 (27,400)		280*(15,000)	314 (15,500)	369 (18,500)	b	20
2-hydroxy, 1-methoxy	229 (30,200)	254 (27,000)	275*(10,000)		369 (53,000)	b	20
2-hydroxy, 3-methoxy	220 (26,400)	245 (34,400)	277*(20,600)	309 (22,700)	355 (19,800)	b	20
2,3-dimethoxy		242 (32,800)	272 (7,900)	305 (11,500)	346 (8,400)	b	21
3-hydroxy, 2-methoxy	225 (28,100)	242 (30,300)	277*(19,400)	313 (20,300)	350 (20,100)	b	21

TABLE 1 (Contd.)

Trioxygenated Xanthones

Xanthone	Absorption maxima					Solvent	Ref.
1,2,3-trimethoxy	243 (41,800)	277 (12,300)	300 (16,300)	335 (7,500)		b	22
1,2-dihydroxy, 3-methoxy	248 (26,800)	270*(12,400)	296 (13,600)	315 (15,200)		b	20
2,3-dihydroxy, 1-methoxy	239 (29,800)	255*(30,400)		313 (14,300)	355 (6,100)	b	20
3-hydroxy, 1,2-dimethoxy	241 (35,200)	280 (8,700)		305 (14,200)	340*(7,100)	b	20
1-hydroxy, 2,3-dimethoxy	242	255*	290	305	362	b	22
1,2,5-trihydroxy	251 (35,980)	267 (37,440)	310*(11,210)	323 (7,900)	406 (9,095)	a	23
1,2,8-trihydroxy	241 (26,000)	265 (36,400)	290 (7,800)	338 (8,900)		b	24
2,8-dihydroxy, 1-methoxy	238 (26,900)	262 (32,800)	290 (5,200)	322 (4,400)		b	24
1,2-dimethoxy, 8-hydroxy	238 (29,900)	260 (35,200)	290 (5,600)	322 (5,000)		b	24
1,2,8-trimethoxy	241 (49,500)		284 (9,100)	311 (9,500)		b	24

TABLE 1 (Contd.)

Xanthone	Absorption maxima					Solvent	Ref.
1,3,5-trihydroxy	266 (28,000)	314 (12,400)	350 (7,400)			b	24
1,5-dihydroxy, 3-methoxy	268 (31,300)	315 (15,000)	355 (4,000)			b	24
1,3-dihydroxy, 5-methoxy	263 (34,000)	312 (16,000)	350 (4,300)			b	24
1-hydroxy, 3,5-dimethoxy	265 (40,200)	306 (19,100)	355 (5,600)			b	24
1,3-dimethoxy, 5-hydroxy	265 (41,800)	304 (18,200)	344 (4,600)			b	24
1,3,5-trimethoxy	266 (54,400)	300 (21,900)	336 (7,300)			b	24
1,3,6-trihydroxy	237 (40,000)	251 (27,000)	297 (10,400)	313 (23,800)		b	25
1-hydroxy, 3,7-dimethoxy	231 (34,710)	259 (38,550)	292 (24,360)		359 (6,780)	b	26
1,3-dihydroxy, 7-methoxy	235 (28,180)	259 (31,620)	311 (13,300)		369 (6,310)	b	26
1,5,6-trihydroxy	251 (39,130)	315.5(6,480)	332 (13,060)			b	27
1,6-dihydroxy, 5-methoxy	243 (39,000)	267* (10,500)	313 (11,300)		357 (8,900)	b	27
1-hydroxy,	236*(37,500)	243 (38,600)	269*(8,500)	305 (11,300)	355 (6,300)	b	27
1,5-dihydroxy, 6-methoxy	236*(26,600)	248.5(35,000)	273 (9,700)	323 (13,700)		b	27

TABLE 1 (Contd.)

Xanthone	Absorption maxima					Solvent	Ref.
1,5,6-trihydroxy		251 (39,130)	315.5(6,480)	332 (15,080)		b	32
1,6,7-trihydroxy	224 (24,400)	253 (25,600)	271 (10,200)	296 (9,900)	373 (10,200)	b	25
2,3,4-trihydroxy	231			317	376	b	21
4-hydroxy, 2,3-dimethoxy	237 (23,400)	256 (32,200)	291 (9,600)	308 (9,300)		b	21
3,4-dihydroxy, 2-methoxy	239 (27,400)	255 (26,700)	285 (5,600)	333 (12,400)		b	21
2,3,4-trimethoxy		246 (33,100)	273 (9,900)	304 (11,200)		b	21
Tetraoxygenated xanthones							
1,3,5,6-tetrahydroxy		253 (48,990)	281 (12,380)	326 (21,380)		a	28
1-hydroxy, 3,5,6-trimethoxy		245 (46,770)	232 (10,720)	314 (23,440)	338 (6,166)*	b	29
1,3,7-trihydroxy, 6-methoxy	239 (48,990)	256 (31,620)		310 (41,300)	362 (9,900)	a	30
1-hydroxy, 3,6,7-trimethoxy	238	256		309	356	b	31
1,3,6,8-tetrahydroxy		252 (28,400)	271 (3,900)	330 (19,200)		a	25

The NMR spectra are free from ambiguities that characterise interpretation of UV spectra but present problems of a different nature which have been, however, resolved to a large extent with the introduction of instruments with more powerful magnets. According to NMR theory the complexity of coupling pattern is related to the ratio $\Delta\nu/J$. When this value is less than 10, i.e. when the chemical shift difference between two protons is small and the coupling constant large the spectrum changes from first order to second. This means that generalisations regarding the multiplicity of signals valid in the case of first order spectra do not hold for second order spectra. One can intuitively still decipher such spectra from the shape of the signal but the task is rendered more complicated by overlapping of the multiplets of other protons. Since chemical shifts are proportional to field strength whereas coupling constants are field independent, $\Delta\nu$ becomes larger as the field strength increases whereas J remains constant. As a result second order effects are minimised and spectra of compounds at 270 MHz are, therefore, much better resolved and can be interpreted according to rules valid for first order spectra. In other words a system of three protons which give rise to complex ABC splitting at 60 MHz will change to AMX system at 220 or 270 MHz. The contrast is brought out strikingly if one compares the spectra of wightianone at 60 and 220 MHz (Fig. 3 and 4). Unfortunately instruments above 100 MHz range are not yet commonly available

and complex second order spectra have to be interpreted with the aid of spin decoupling, solvent and lanthanide induced shifts.[33]

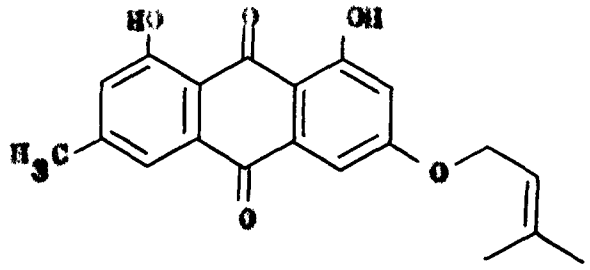
The protons of the unsubstituted benzene ring give rise to a sharp singlet at 2.70. The introduction of electron donating or withdrawing substituents shifts the signal upfield or downfield respectively and Ballantine and Killinger, who studied the spectra of a number of aromatics, found these shifts to be additive.[9] This fact enables one to infer to some extent the number and position of such substituents with respect to a given aromatic proton (Table 2).

TABLE 2

Substituents shielding values measured in ppm from benzene. 10% solution of benzene in $CDCl_3$ absorbs at 2.70. (negative sign indicates a shift to lower field).

Substituents	ortho	meta	para
-OH	0.45	0.10	0.40
-O-alkyl	0.45	0.10	0.40
-O-CO-R	0.20	-0.10	0.20
$-NH_2$	0.55	0.15	0.55
$-CH_3$	0.15	0.10	0.10
$-CH_2-$	0.10	0.10	0.10
-CH	0.00	0.00	0.00
-CH=CH-R	-0.10	0.00	-0.10
-CHO	-0.65	-0.25	-0.10
-CO-R	-0.70	-0.25	-0.10
-COOH (R)	-0.30	-0.25	-0.20

As an example of the validity of their generalisations the authors have compared the observed and calculated values of the chemical shifts of some aromatic protons of more complex molecules. Thus for the quinone[34] (X) the calculated value for protons at C-4 and C-2 are 2.95 and 3.30 respectively which is close to the observed values 3.00 and 2.50 respectively. The NMR spectra of xanthones are also subject to generalisations discussed above and table 3 collects chemical shifts of protons of the xanthone nucleus.



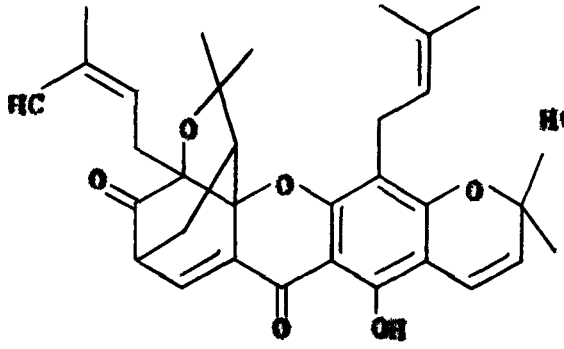
(X)

TABLE 3

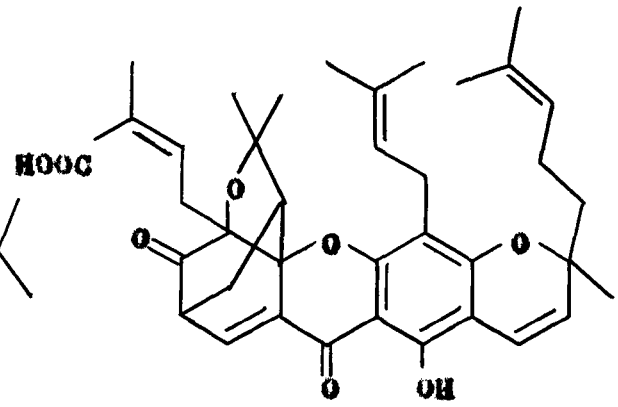
Proton	Calculated Chemical Shift	Observed Chemical Shift
1-H	2.10	1.90
2-H	2.55	2.75
3-H	2.70	2.65
4-H	2.90	2.85

Prenylated Xanthones

Like coumarins and flavonoids many xanthones carry isoprenoid residues which are present either as side chains or are cyclised with adjacent hydroxyls to add an additional six-membered heterocyclic ring to the xanthone nucleus. A striking feature of naturally occurring xanthones is that isoprene residues are of much more frequent occurrence than is the case with flavonoids. Indeed the majority of xanthones isolated during the last ten years are prenylated and their prenyl residues are found frequently cyclised with the aromatic ring to give rise to structures reminiscent of the products of photochemical reactions. The earliest examples of this class of compounds are the morellins e.g. morellin[35] (I) and gambogic acid[36] (II). Another point worth stressing here is that to date only a few furanoxanthones have been reported whereas furanoflavones and furanocoumarins are of rather common occurrence. The 3,3-dimethylallyl side chain may be present at any position but are rather more commonly attached to that benzene ring which has phloroglucinol type of oxygenation and this makes it difficult to distinguish between 2 and 4 prenyl compounds because the chemical shifts of 2 and 4 protons are almost identical.



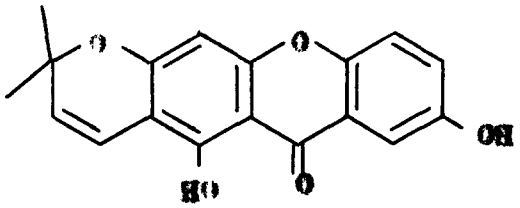
(XI)



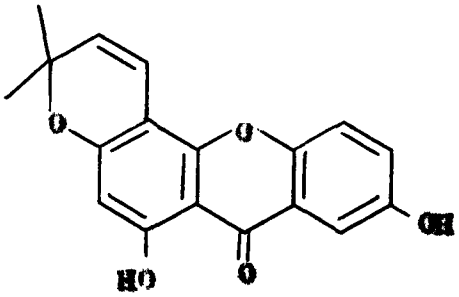
(XII)

A problem of the same kind is encountered in the structure elucidation of xanthones in which the 3,3-dimethylallyl side chain has undergone oxidative cyclisation with the adjacent hydroxyl. Thus osajaxanthone[37] may have either of the two possible structures (XIII) and (XIV) since the singlet of the aromatic proton at 3.69 can be assigned to either the 2 or 4 proton. In this case the linear structure (XIII) was favoured on the basis of positive Gibbs test given by its monomethyl ether but even when this test is carried out under spectroscopic control its validity is not all that certain. It is fortunate therefore, that another method exists which neatly distinguishes between the two alternatives. Introduced by Merlini et al[38] in 1967 it has been applied by now to a large number of compounds and so can be said to have stood the test of time. The resonance of the α -proton of the chromone ring appears at about 3.69 in

compounds without an adjacent phenolic hydroxyl. Presence of such a hydroxyl, for reasons which are not yet certain, produces a paramagnetic shift to about 3.29 which is offset by acetylation so that one observes an upfield shift of the doublet of this proton of about 0.28 when the spectra of free phenol and the acetate are compared. The doublet of the chromene β-proton experiences at the same time a downfield shift of smaller magnitude.



(XIII)

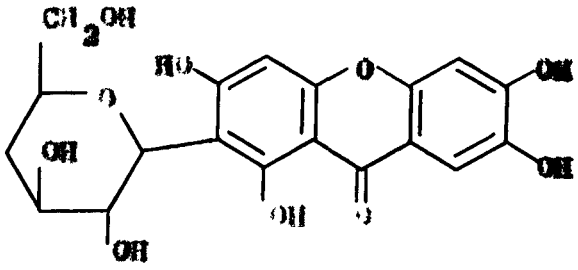


(XIV)

Xanthone glycosides

The prenyl residues are not the only non-aromatic entities attached to the aromatic rings. A large number of polyphenols have been isolated as C- or O-glycosides, O-glycosides being much more common than the C-glycosides. Elucidation of structures of these glycosides requires the establishment of the nature of the sugar moiety and its point of attachment to the polyphenol. The first xanthone glycoside, mangiferin[39] was initially wrongly considered to be an O-glycosyl compound. It was later shown to

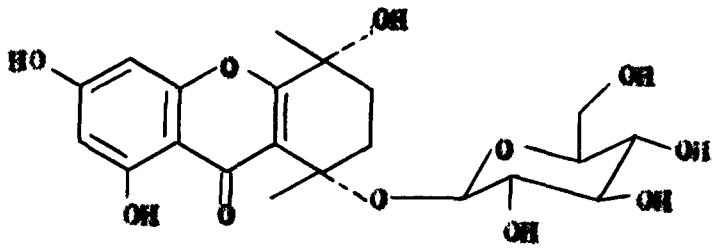
have structure (XV). Since then, and specially during the last few years, a number of xanthone glycosides have been isolated.[40] Of particular interest here is that such glycosides appears to be distributed mainly in three genera of Gentianaceae family; Swertia, Gentiana and Canscora.[40]



(XV)

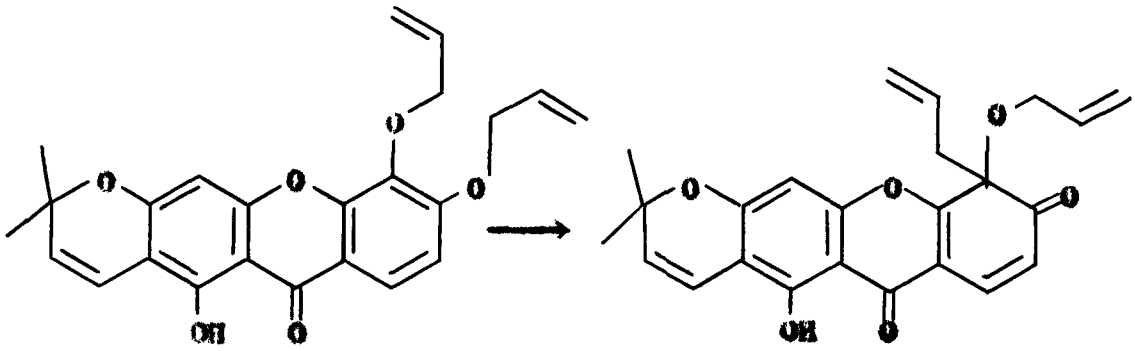
Tetrahydroxanthones

Tetrahydroxanthones are still comparatively rare. The glycoside compestroside (XVI) reported by Hostettman et al[41] contains the tetrahydroxanthone nucleus in its simplest form. The disappearance of aromatic character of one of the two benzenoid rings of xanthones is a feature also of the complex compounds like morellins (XI) and gambogic acid (XII). In morellin one of the three prenyl side chains has undergone fusion with the aromatic ring to give rise to a 2,2-bicyclohexane system. The preponderance of such xanthones is noteworthy since similar compounds have not been encountered in chromones and flavonoids.

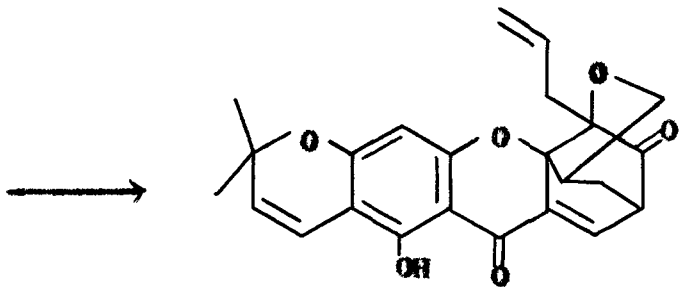


(XVI)

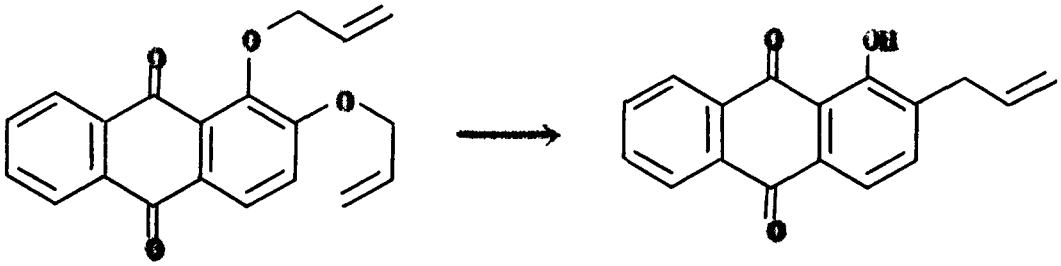
In a major advance towards an understanding of the formation of such complex systems in nature, Gillinam and Scheinmann[42] have demonstrated that 5,6-diallyl ether of jacareubin (XVII) when heated in boiling decalin for 14 hours gives rise to jacareubin with a bridged cyclohexane system (XVIII) similar to that of morellins. This reaction must be interpreted in the light of earlier findings that the side chain of 6-allyloxy xanthones migrates preferentially to the 5 position during Claisen rearrangement. The subsequent step is the Diels-Alder addition of the side chain double bond to the diene system (XIX) generated in the reaction. In spite of the severity of conditions the biosynthesis reaction may follow a similar course. It is interesting that in a recent communication the same authors report the elimination of allyloxy side chain from 1,2-diallyloxy anthraquinone (XX) under the conditions of claisen rearrangement.[43]



(XVII)　　(XIX)



(XVIII)

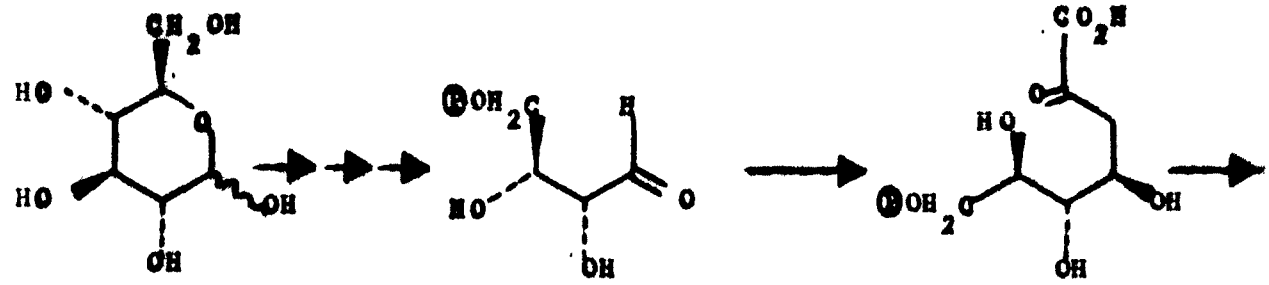


(XX)

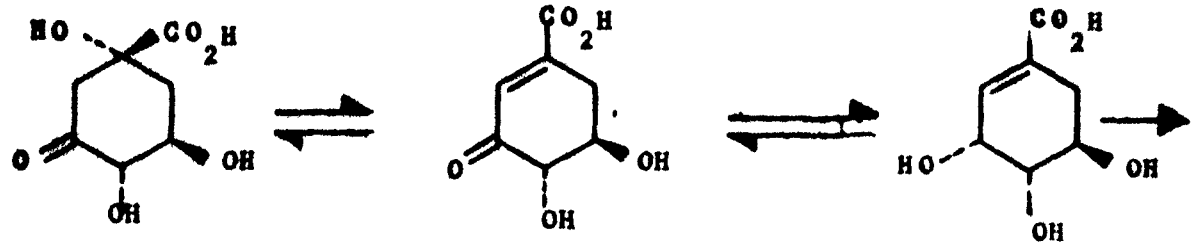
Biogenesis of Xanthones

The aromatic compounds occurring in nature present a bewildering structural diversity with piperonal, orsellinic acid and simple phenols representing one end of the spectrum, griseofulvene, aflatoxins and morellins the other. In spite of these structural variations they can be classified in terms of two basic metabolic pathways, one starting from carbohydrates in which the key intermediates are shikimic acid, phenylalanine and cinnamic acid (Scheme 1), the other from acetic acid in which polyketides of varying chain lengths and folded appropriately for the formation of the target molecule are the immediate precursors. While it is not possible to establish which of the two pathways is operative in the biogenesis of a naturally occurring aromatic without recourse to detailed studies with labelled compounds the origin of most aromatic rings can be inferred from the sites of oxygenation. Thus it was early concluded that ring A of flavonoids is derived from acetate because it invariably has phloroglucinol oxygenation and ring B from carbohydrates as it usually carries oxygens at 3',4' positions. The subsequent detailed investigations by Grisebach provided experimental justification of these assumptions. Similarly another related group of naturally occurring compounds, the coumarins, are derived from umbelliferone which is

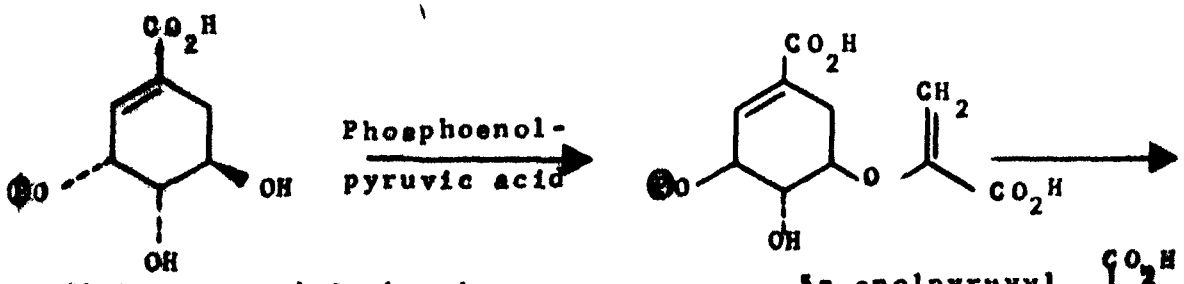
SCHEME- 1



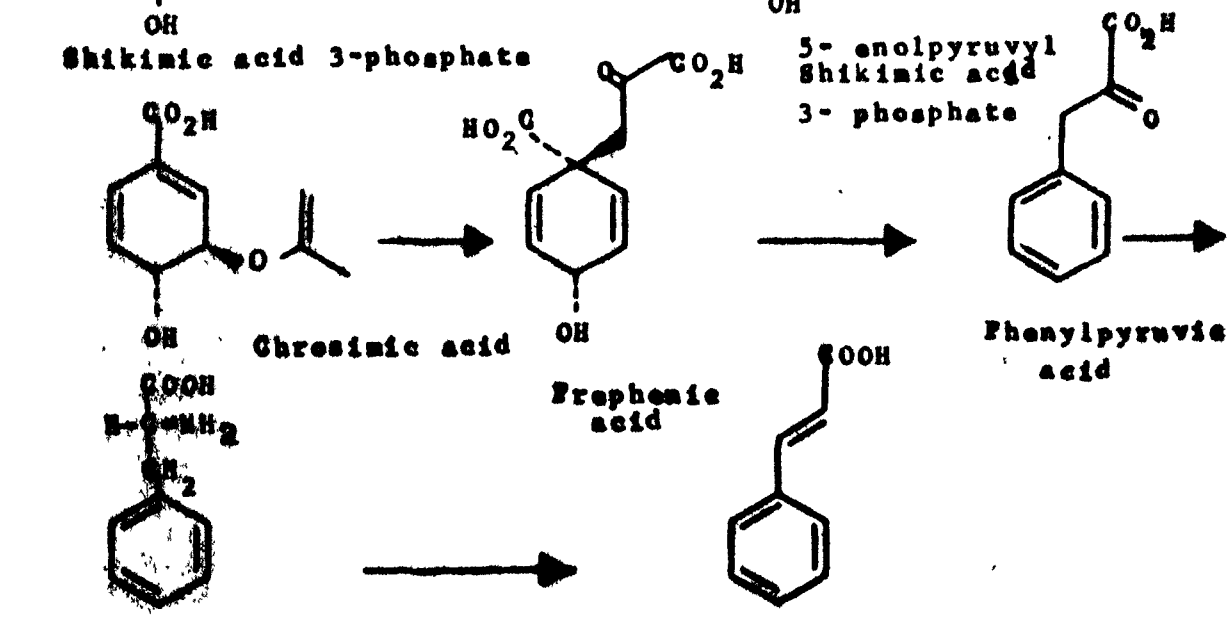
D- glucose Erythrose-4-phosphate



3- dehydroquinic acid 3-dehydroshikimic acid Shikimic acid



Shikimic acid 3-phosphate 5- enolpyruvyl Shikimic acid 3- phosphate

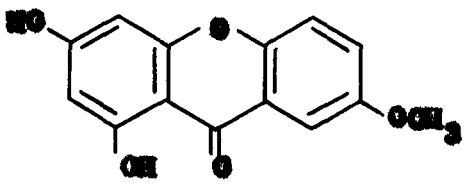


Chrosimic acid Prephenic acid Phenylpyruvic acid

L- Phenylalanine Cinnamic acid

obviously the product of intra-molecular cyclisation of p-hydroxy cinnamic acids.

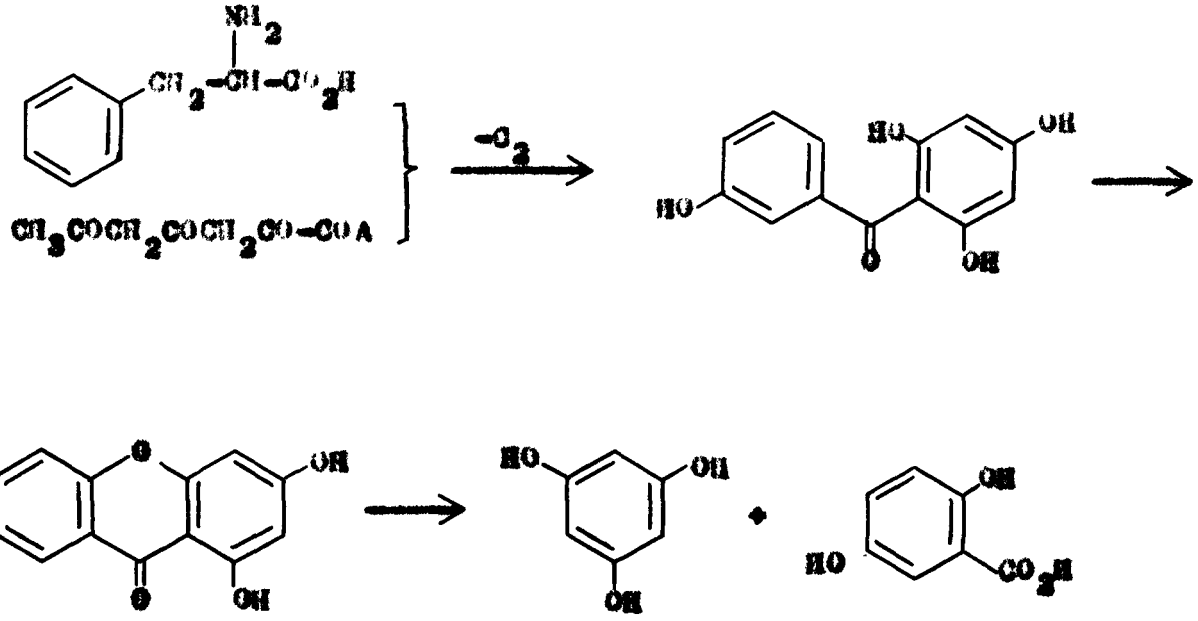
The xanthones isolated from higher plants show considerable variation in their hydroxylation pattern but one of the two rings usually has phloroglucinol substitution and its origin from acetate is, therefore, to be expected. Recent work with labelled compounds is in harmony with the assumption that xanthones, like flavonoids, are of mixed acetate/shikimate origin. The acetate origin of the phloroglucinol ring of gentisin (XXI) was demonstrated by Floss and Vettig[44] in 1964 and the involvement of shikimic acid via phenylalanine was brought out by the work of Gupta and Lewis.[45] Barton's suggestion[46] that xanthones are produced by oxidative coupling of dioxygenated benzophenones received experimental support from the work of Barton and Scott[47] and while in vitro studies by Lewis and co-workers[48-50] provided further evidence for the correctness of this view, an extension of the biogenetic scheme became necessary when anthrones and anthraquinones were shown to be precursors of some fungal xanthones.



(XXI)

Another significant point to emerge from the work of Gupta and Lewis was that during the biogenesis of gentisein (XXII) phenylalanine must have suffered the loss of two carbon atoms since the labelled carbon was present in the xanthone only if 3-^{14}C phenylalanine was employed (Scheme 2). In keeping with this finding, degradation showed that only gentisic acid contained the incorporated radioactivity. It is also worth noting in this context that phloroglucinol itself is not incorporated into xanthones and the linking of the shikimic acid derived ring to the frame-work of acetate carbons, therefore, occurs prior to cyclisation of the polyketide chain to phloroglucinol.

Scheme 2

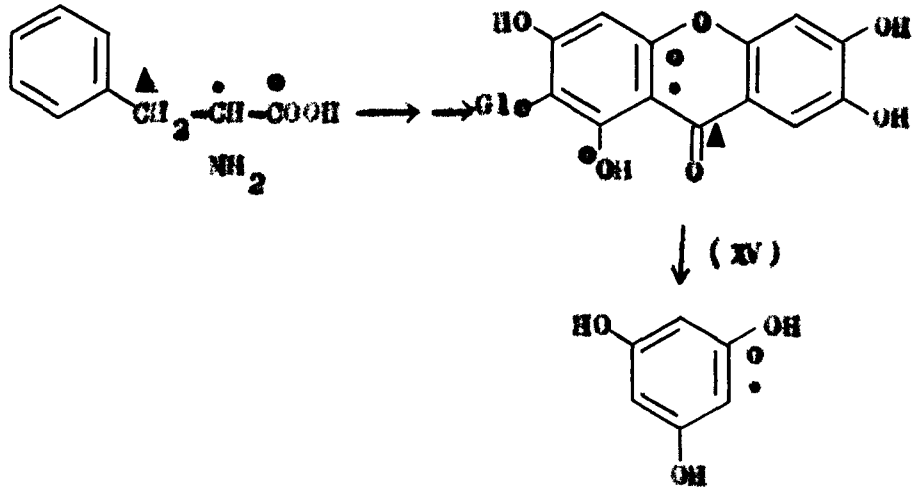


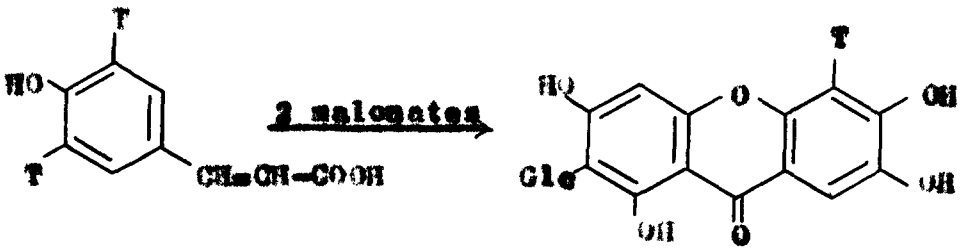
(XXII)

The loss of two carbon atoms is not, however, a prerequisite for incorporation of phenylalanine into xanthone since Fugita and Inoue[51] found that C-1, C-2 and C-3 labelled cinnamic acid was efficiently incorporated into mangiferin (XV) in Anarrhena asphodeloides. If 1 or 2-^{14}C labelled phenylalanine or p-coumaric acid was used, the labelled carbon, as shown by degradations, appeared in the phloroglucinol ring and if 3-^{14}C labelled compounds were used, the label was located entirely in the heterocyclic ring.

When 3,5-ditritiated 2-^{14}C p-coumaric acid (XXIII) was fed to the plant the T/^{14}C ratio in the isolated mangiferin was nearly the same as in the p-coumaric acid precursor when adjustment was made for the loss of one T through hydroxylation. Labelled malonic acid was also efficiently incorporated and as expected the activity was now located in the phloroglucinol ring (Scheme 4). The conclusion is obvious that mangiferin is derived from phenylalanine or para-hydroxy cinnamic acid through condensation with two malonate units and that it is formed through condensation of a C_6, C_3 rather than a C_6, C_1 unit.

Scheme 3



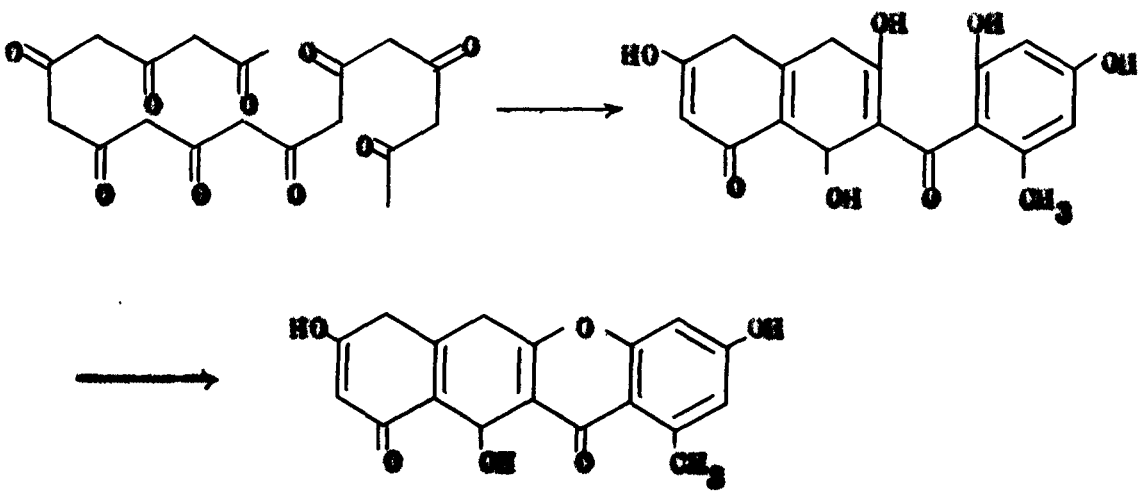


(XXIII) (XV)

There can, thus, be no doubt that xanthones in higher plants are derived from a confluence of two metabolic pathways though the stage, and the exact manner in which, the two units get linked together may differ from case to case. As against this all work on the biogenesis of fungal xanthones shows that they are derived solely from acetate.

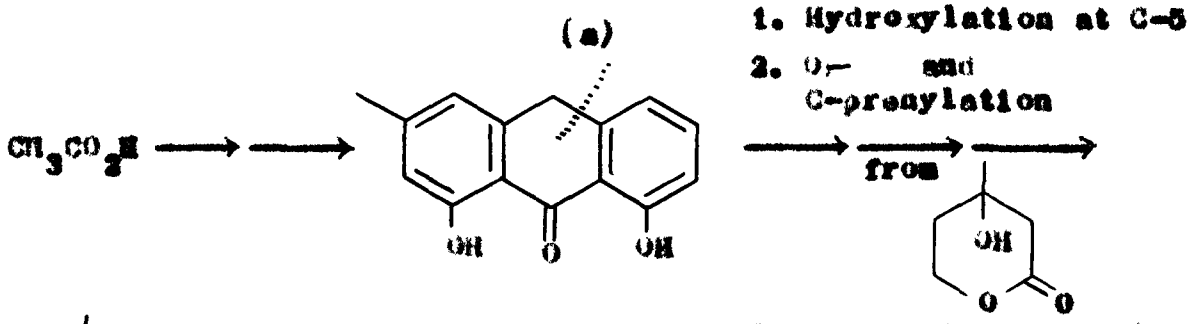
The biogenesis of fungal xanthones proceeds through two distinct pathways. In one a singly folded polyketide chain undergoes aldol condensation to a benzophenone which then suffers loss of water to give a xanthone. This is illustrated by Scheme 4 suggested for the biogenesis of bisaverine[52] (XXIV). In the other, condensation through a different type of folding leads to an anthrone/anthraquinone intermediate which undergoes bond scission as shown and the resulting benzophenone, after a 180° rotation which places the hydroxyl groups concerned in cyclisation close to each other, undergoes cyclodehydration to xanthone. This scheme was put forward by Holkar and co-workers[53] to account for the results obtained by them on the biogenesis of fajixanthone (XXV) and are illustrated in Scheme 5. Fajixanthone was initially isolated by Kamal et al[54] and given biogenetically unsound structure (XXVI) which was corrected to (XXV) by Holkar and co-workers.[55]

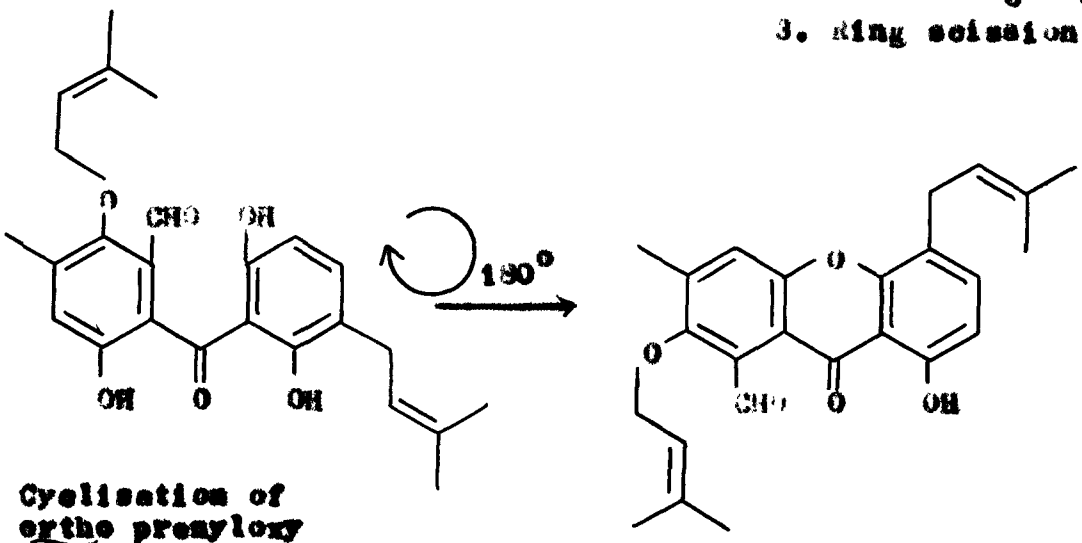
Scheme 4

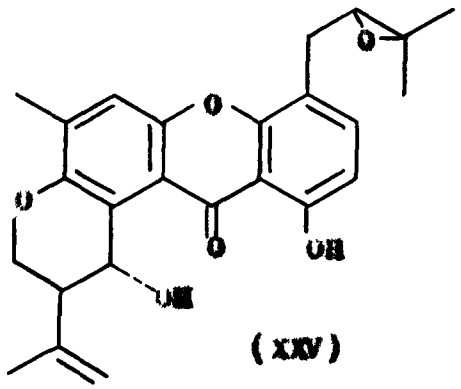


(XXIV)

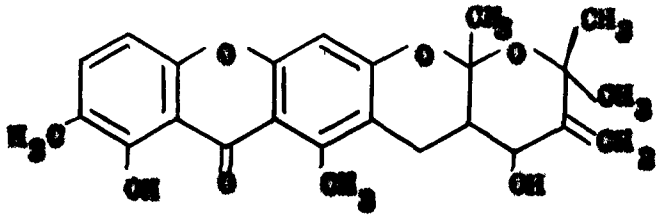
Scheme 5







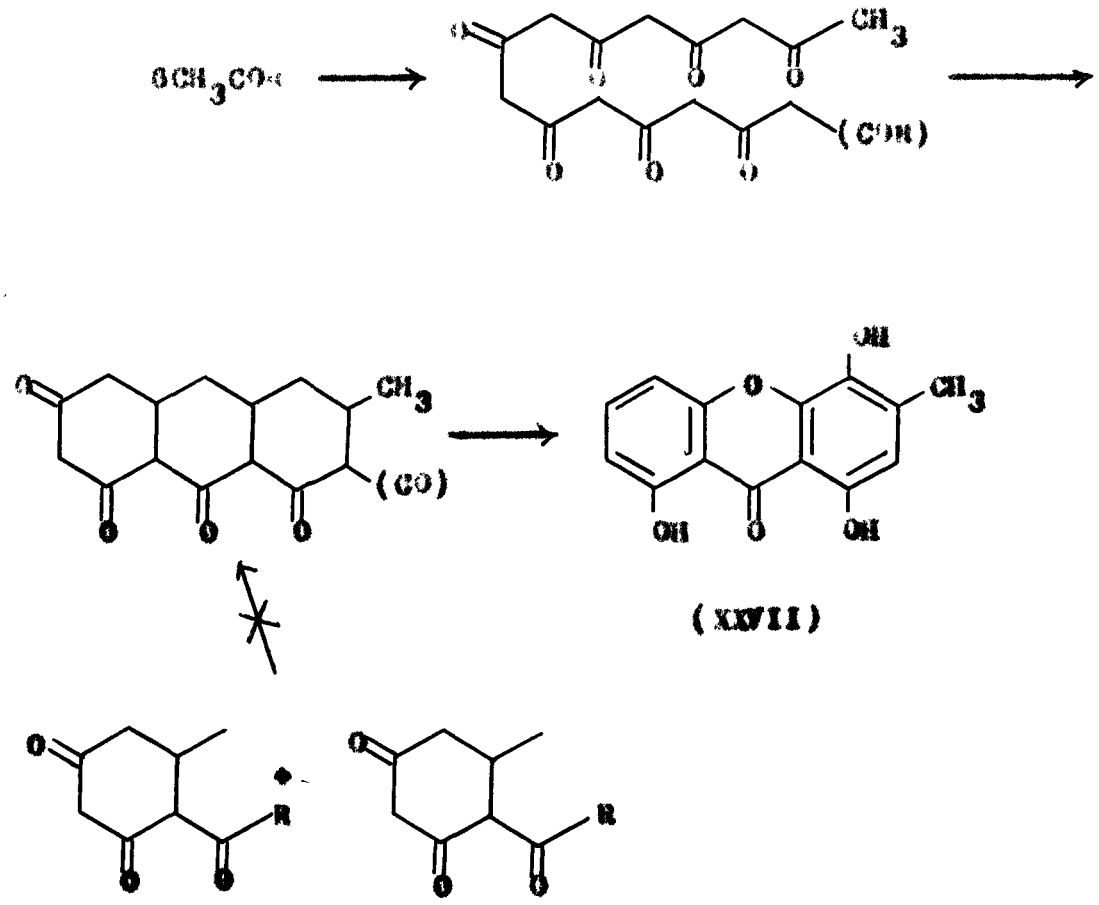
(XXV)



(XXVI)

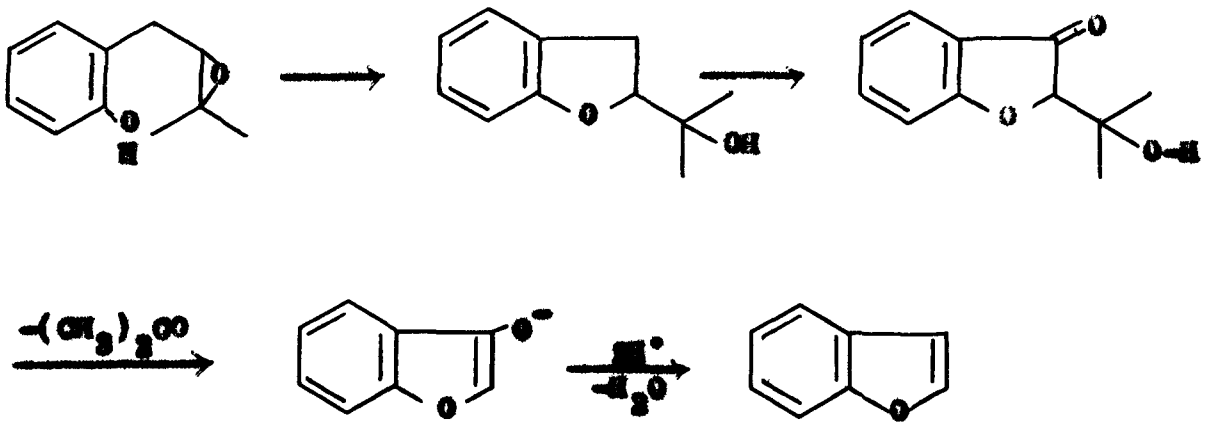
There are perhaps other possibilities which will come to light as more work is done in this area but the only detailed study apart from those cited above confirms to the existing pattern and the biogenesis of revenellin[56] (XXVII) occurs also through an anthrone intermediate and does not involve the condensation of two precyclised polyketide units (Scheme 6).

Scheme 6



The C_5 side chains which are present either as such or have undergone oxidative cyclisation with adjacent hydroxyl are derived from activated acetic acid through mevalonolactone. The oxidative cyclisation to chromene postulated by Ollis[37] has been realised *in vitro* with the help of DDQ in benzene.[38-64] The furan ring as stated earlier is of comparatively rare occurrence in xanthones though very common among coumarins. These are also of isoprenoid origin and are believed to be derived through initial epoxidation of the side chain followed by anion attack and elimination of acetone[65,66] (Scheme 7).

Scheme 7



DISCUSSION

CALOPHYLLUM WIGHTIANUM T. ANDERS

STRUCTURE OF WIGHTIANONE

Calophyllum wightianum (N.O. Guttiferae) is a moderate sized tree occurring in the evergreen forests of western ghats from Mysore to Travancore up to an elevation of 1000 feet and on the banks of rivers and streams.[67] Even in its natural habitat, however, Calophyllum wightianum is a rare tree and whereas one comes across an abundance of Calophyllum inophyllum, only one plant of Calophyllum wightianum was sited during a three week collection tour of the forest regions of Goa on the south-west coast of India. The scarcity of this plant is also reflected in the literature survey which shows only cursory work to have been done on it[68] whereas Calophyllum inophyllum has been studied both in India and abroad.[69,70]

Extraction of the heart wood of Calophyllum wightianum collected from Goa in April, yielded a light yellow compound, of which, after extensive chromatographic purification and crystallisation, about 500 mg was obtained. It was sharp melting and gave a single spot on TLC plates and could, therefore, be considered pure. Repeated elementary analysis gave fairly close values for carbon and hydrogen but the formula worked out from these did not have the molecular weight indicated by the mass spectrum. Thus for C=73.96, H=8.05%, on the basis of only one

oxygen in the compound, one has C=5.55, H=7.25, O=1. Multiplication by four leads to C=22.2, H=29.0, O=4 on the basis of which one can develop two molecular formulae, $C_{22}H_{28}O_4$ or $C_{22}H_{30}O_4$ which require C=74.13, H=7.96 and C=73.71, H=8.44% respectively. The percentage error in carbon and hydrogen is thus within the accepted range but the molecular weight, 356 or 358 is incompatible with $M^{+\cdot}$ at m/e 450. The other possible combinations are $C_{27}H_{36}O_5$, $C_{27}H_{38}O_5$ which correspond to C=73.60, H=8.20 and C=73.27, H=8.65% respectively. But the molecular formula $C_{27}H_{36}O_5$, though it gives the best fit with experimental values, again does not have the required molecular weight. If the m/e 450 peak is attributed to an impurity, the absence of any peak at m/e 440/442 corresponding to the above formulae is not understandable. Thus the discrepancy between the required molecular weight and that shown by the mass spectrum, though much less with these formulae, remains.

To decide about the number of oxygens in the molecule, the IR spectrum (Fig. 1) was consulted at this stage. The spectrum shows broad hydroxylic absorption at 3110 and well defined carbonyl bands at 1700 and 1645 cm^{-1}. The break in the upward flow of the line marking the 1645 cm^{-1} band is suggestive of one more carbonyl group in the molecule but is more likely to be due to the absorption of a conjugated double bond; compare, for instance, ν C=C at 1630 cm^{-1} in conjugated δ -lactones.[71] Taking the stretch and depth of the hydroxylic band into consideration two oxygens can be allotted to hydroxyl functions. The

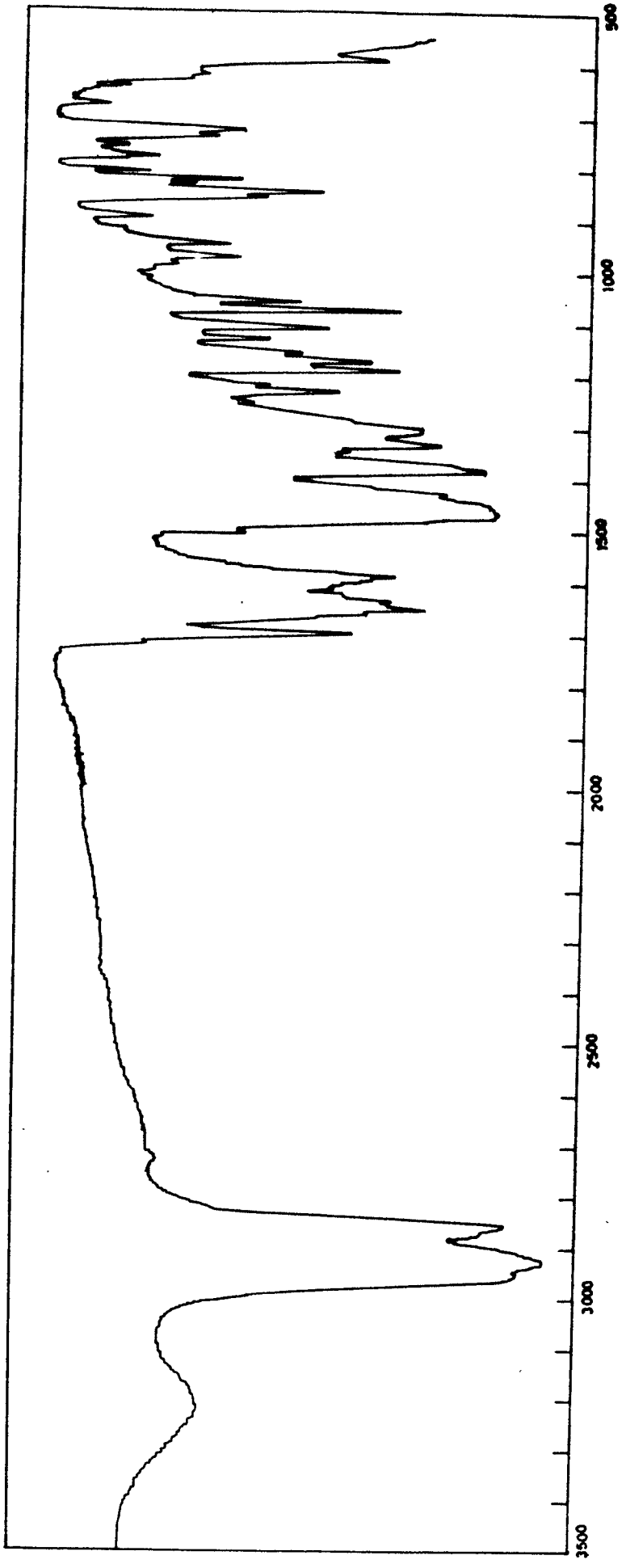


FIGURE- 1

two carbonyl groups evident from 1700 and 1645 cm^{-1} bands account for two more oxygens. While the 1700 cm^{-1} band can only result from an open chain or six membered ring carbonyl, the band at 1645 cm^{-1} may be interpreted to arise either from a chelated benzoyl group, which must be present since the compound gives a positive ferric reaction, or a benzo-γ-pyrone moiety such as in flavones, isoflavones etc. On the basis of the above considerations and the molecular formula $C_{27}H_{36}O_5$ the remaining oxygen is best assigned to an ether function such as chromones, flavones, isoflavones etc.

The phenolic nature of two hydroxyls and chelation of one of these with the carbonyl is evident from the formation of a monoacetate. The NMR of monoacetate (Fig. 3) shows one acetate methyl at 7.70 and OH proton in the offset. Similarly treatment of the compound with diazomethane gave monomethyl ether which retained the positive ferric reaction of the starting material. While it would be pointless to consider other features of the NMR spectrum of the monoacetate, the shift of 0.25 to lower field of the singlet of the aromatic proton shows that it is next to the hydroxyl which is acetylated as acetylation of a para hydroxyl normally results in larger paramagnetic shift.[108]

The UV spectrum of the compound (Fig. 2), which shows maxima at 230, 268, 300, 330 (sh)nm, when compared with the set of spectra of flavones and isoflavones given in "Systematic Identification of Flavonoid Compounds" by Mabry, Markham and

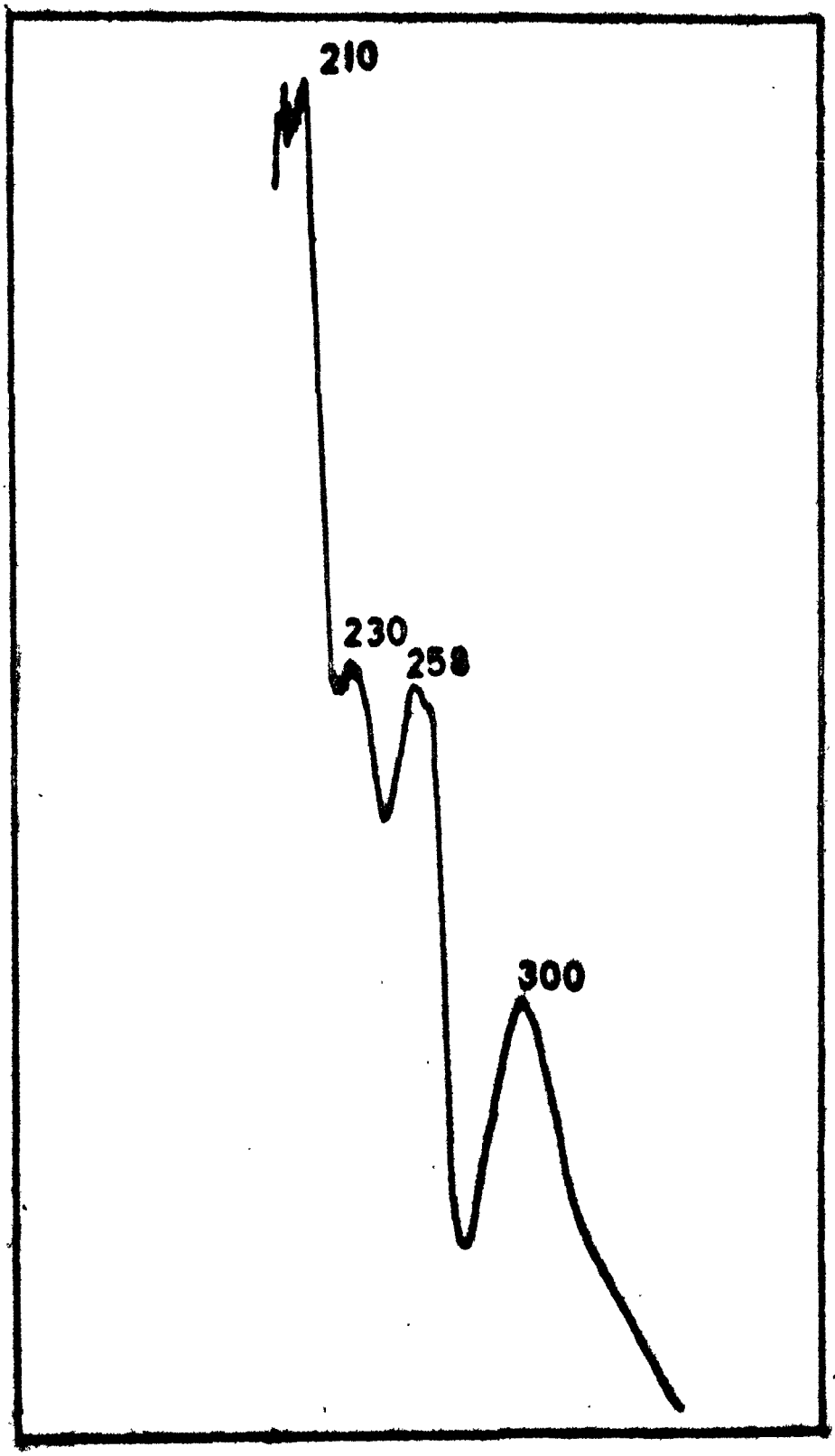


FIGURE- 2

Thomas[72] showed some resemblance to the spectra of isoflavones but the correspondence was not close enough to justify assumption of an isoflavone nucleus. Since xanthones like isoflavones are basically chromones a molecule of this type is also possible. Comparison with the spectra listed in table 1 is, however, not of much use because of the wide variations in the absorption of differently substituted xanthones. The strong absorption at 230 nm, however, demands a benzoyl chromophore and as such the parent nucleus of the compound may be that of isoflavones, chromones and since there is little interaction across the carbonyl group between the two benzene rings, xanthones.

The combined evidence of the IR and UV spectra, therefore, shows that the molecule has five oxygens and hence the formula $C_{22}H_{28-30}O_4$ can be safely eliminated. In spite of the evidence in its favour, $C_{27}H_{36}O_5$ also does not qualify as the molecular formula of the compound for reasons which will become apparent in course of the discussion but specifically because of its incompatibility with the molecular weight shown by the mass spectrum. The only permissible formula if the peak at m/e 450 is assigned to the molecular ion, is $C_{28}H_{34}O_5$. This is not in as good accord with the C, H values revealed by elementary analysis (C=74.64, H=7.61%) as $C_{27}H_{36}O_5$ but traces of impurities can cause variations of this extent. It was hoped that the number of protons shown by the NMR spectrum would offer decisive evidence to permit a choice to be made between these two molecular formulae. As it

turned out, the NMR spectrum instead of settling the issue adds to the confusion.

The 60 MHz NMR spectrum (Fig. 3) has poor resolution and is not of much help in devising a possible structure for the compound. The 220 MHz spectrum (Fig. 4) is well resolved and integrates for a total of 40 protons as against 36 or 38 required by the above formulae. The extra protons are to be expected, of course, if an impurity is contaminating the sample but all the signals in the NMR spectrum integrate for a whole number of protons which goes against the assumption of the presence of an impurity. The smallest rise of the integral is equal to seven units and this can be reasonably assumed to represent one proton. The rise of the integral over all other signals is either a multiple of this or very close to that e.g. 42 units over the highest field singlet which must therefore be attributed to two methyls and fifteen each over the multiplets between 6.80 and 7.40 each of which must be attributed, on this basis, to two protons. Working in this way, and including the exchangeable proton in the offset, one has in all a total of forty protons. The confusion with regard to the true molecular formula, therefore, persists.

It is clear from the NMR spectrum that the compound carries several isoprene side chains. Excluding the singlet of the aromatic proton at 8.66 which rises above the broadish signal of one phenolic hydroxyl, there are two multiplets at low field which can be attributed to the three olefinic protons of the side

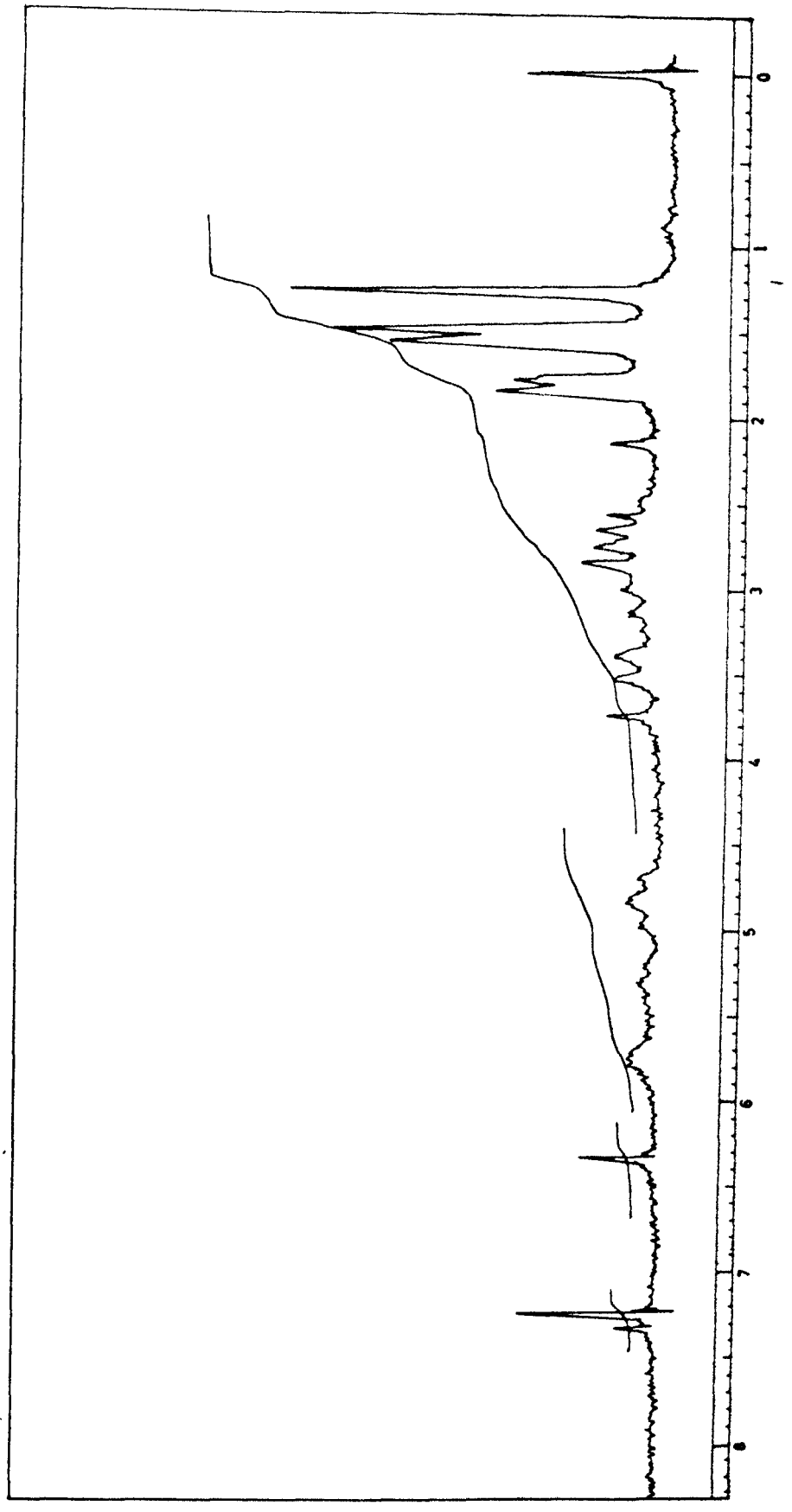


FIGURE- 3

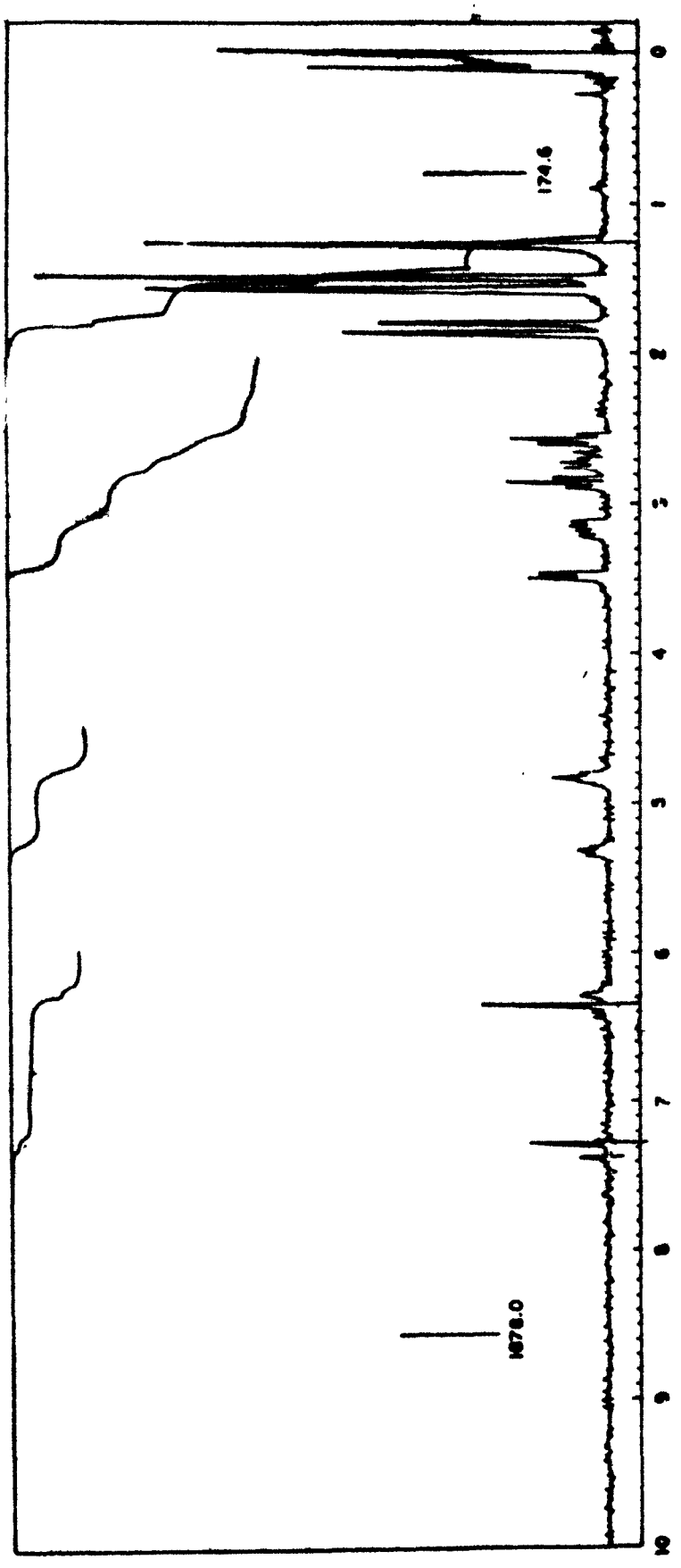


FIGURE- 4

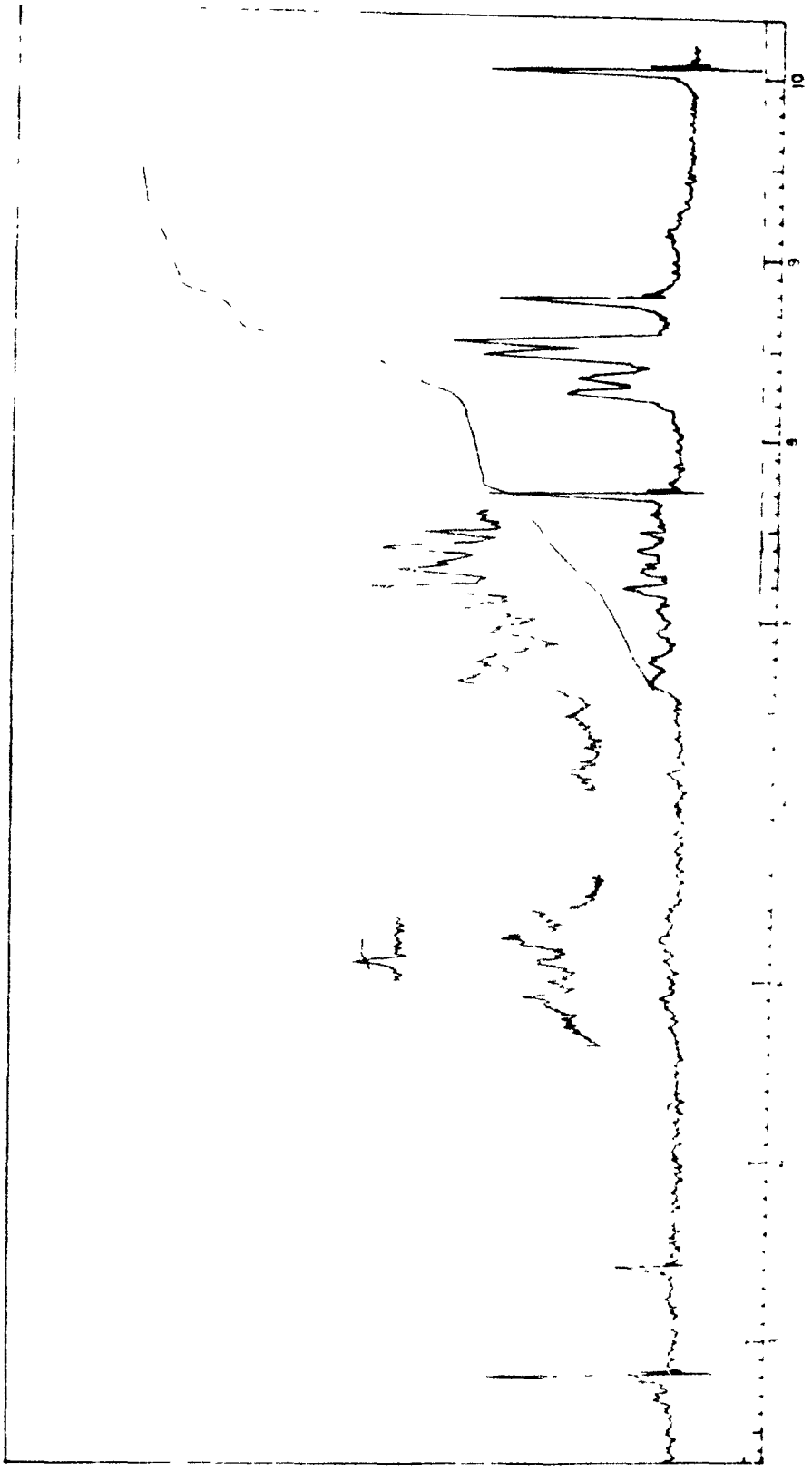


FIGURE- 5

chains. The triplet at 4.65 is obviously due to the olefinic proton of a γ,γ-dimethylallyl group attached to the benzene ring, the corresponding methylene doublet is clearly seen at 6.50. These values are fully in accord with those reported in literature e.g. for auriculasin,[89] auriculatin[89] and auriculin.[90] The part structure (XXVIII) is thus recognisable and the rest of the molecule must carry at least two γ,γ-dimethylallyl residues if the 2H multiplet at 5.15 and methyl singlets are to be accounted for. These two C5 units can not be attached to a benzene ring either separately or as the geranyl side chain (XXIX) since there is only one benzylic methylene, the one responsible for the doublet at 6.50.

The presence of three isolated double bonds in the compound was confirmed through hydrogenation which occurred readily over palladium charcoal and supplied a hexahydro derivative ($M^{+\cdot}$ 456). The carbonyl bands of the product appear at approximately the same value as in the IR spectrum of the parent compound so that neither the carbonyl nor the double bond conjugated with one of these is touched during hydrogenation. Comparison of the NMR spectrum of the hydrogenation product (Fig. 6) with that of the starting material offers conclusive evidence of the presence of three prenyl side chains, two attached to sp^3 carbon/carbons and one to the aromatic ring since the multiplets of the methine protons near 5 τ as well as the broad doublet of the benzylic methylene are not present in the NMR spectrum of the hydrogenation product. The signals of the olefinic methyls are, of course, replaced by others

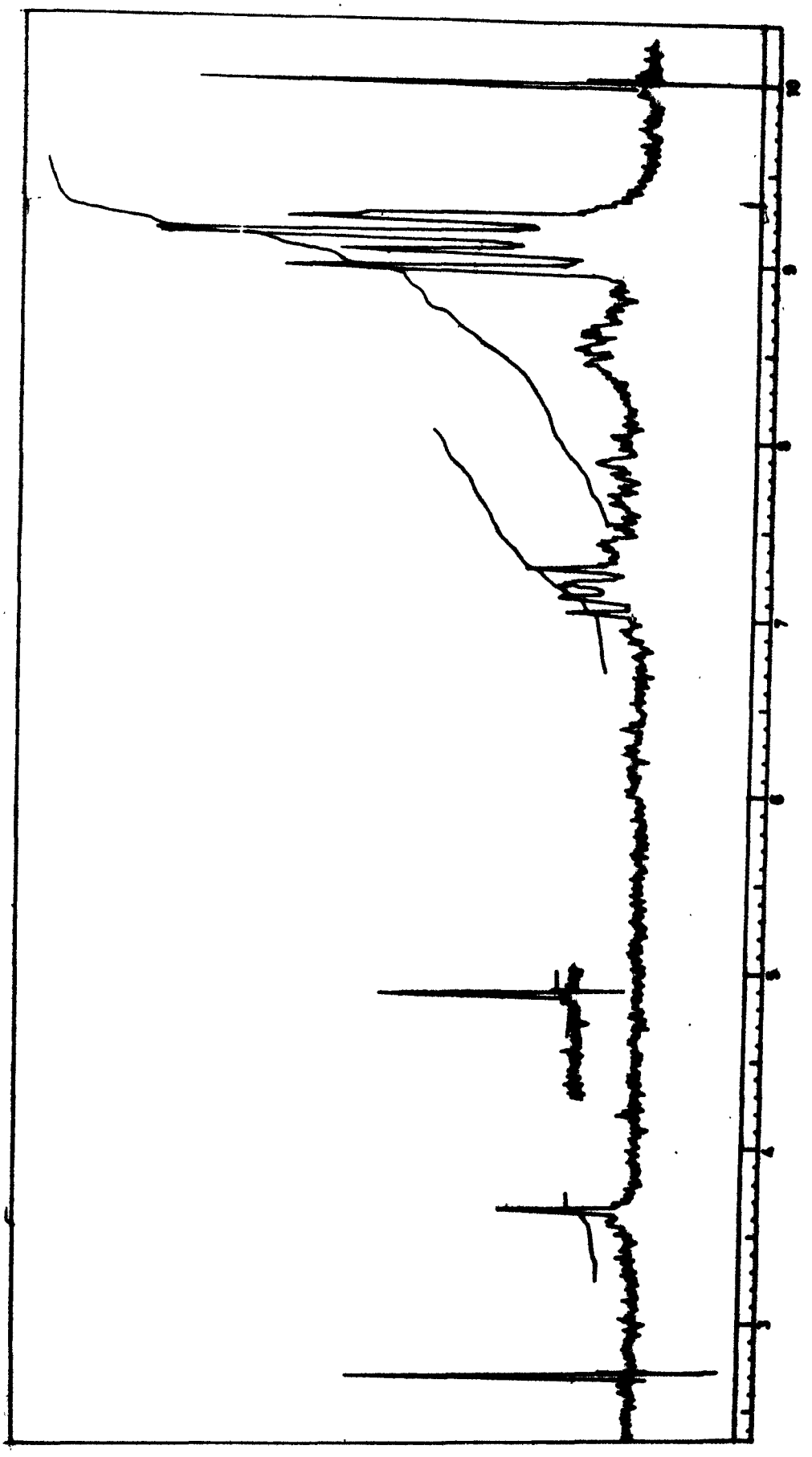
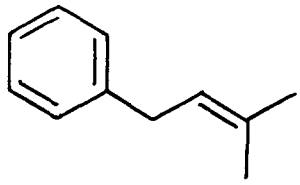
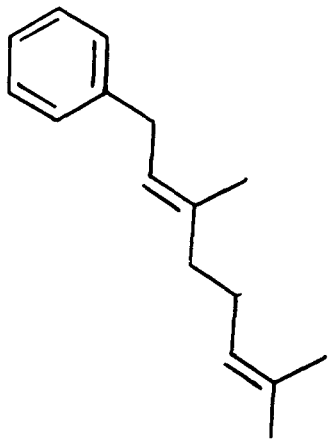


FIGURE- 6

at higher field.

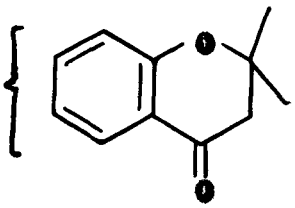


(XXVIII)



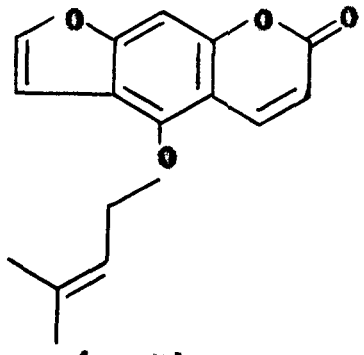
(XXIX)

This leaves the 6H singlet at 8.80 in the spectrum of parent compound still to be accounted for. It is at too high a value to be attributed to olefinic methyls and the absence of coupling allows only methyls on quaternary carbons. One possible solution which is also biogenetically plausible is to allot the singlet to the gem-dimethyls of a chromanone (XXX). The total proton count in this way adds up to 40 whereas the molecular formula consisted with $M^{+\cdot}$ at 450 permits only 34.

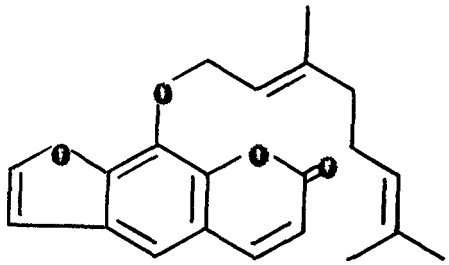
Substituents 

(XXX)

Discrepancies of this nature in proton count sometimes result when what is regarded as a single entity is in fact a mixture of two compounds having closely related structures. Such mixtures are often inseparable on chromatostrips and a case in point is the mixture of imperatorin (XXXI) and 8-geranyloxy psoralen (XXXII) obtained from Heracleum sandiecum.[73] Had the actual ratio of this mixture been 1:1 instead of 2:1 the NMR spectrum would have offerred no indication that it was a mixture. Extending this line of reasoning to the problem in hand did not lead to any reasonable structural isomers and yet the NMR spectrum offers no evidence of an impurity to which the molecular ion at m/e 450 could be assigned.



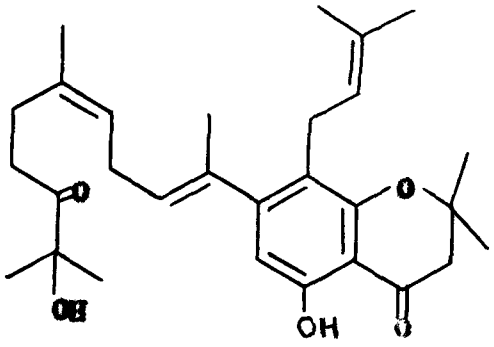
(XXXI)



(XXXII)

Another possibility which has to be looked into is that the 450 peak does not belong to the molecular ion but arises through loss of water from it. One would have to invoke the presence of a tertiary hydroxyl to justify this assumption. The

only molecular formula possible on this basis is $C_{28}H_{40}O_5$ and loss of H_2O from this gives $M^{+\cdot} - H_2O$ at m/e 450 corresponding to $C_{29}H_{38}O_4$. It is difficult however, to put together a carbon skeleton on the basis of this molecular formula. Structures such as (XXXIII) which, to some extent, has the required spectral features, do not stand closer scrutiny. In fact all attempts to develop a structure which would accommodate all the forty protons lead to absurd propositions. Any doubt as regards the validity of the 450 peak was removed when a high resolution mass spectrum was obtained. This showed $M^{+\cdot}$ at 450.2493 which is in close agreement with the required value 450.2406 for $C_{28}H_{34}O_5$.



(XXXIII)

Since the accurate mass settles the molecular formula $C_{28}H_{34}O_5$, the only explanation left is that one 6H singlet, most probably the one at highest field, is due to an impurity and because there is no apparent evidence of signals of other protons of this impurity it has to be assigned to a molecule like that of

a long chain hydrocarbon. This hydrocarbon, if present in a proper molecular ratio, would give rise to a signal integrating exactly for six protons. The impurity could originate from the packing of solvents used for chromatography or could be present in the plant itself. It is, however, inexplicable how a hydrocarbon sticks to the molecule in such a way that its proportion does not vary after repeated crystallisation. Clearly a clathrate is indicated. It is interesting that reports of earlier investigations on the plant mention the presence of palmitic, stearic acids etc.[69] in the seeds. Fatty acids are known to form inclusion complexes with urea and could do so with other compounds. The idea of the presence of fatty acids as an impurity is further supported by careful and closer scrutiny of the 220 MHz NMR spectrum of the compound (Fig. 4) which shows that there is, just clear of the noise, a triplet at 9.12 that can be ascribed to a terminal methyl group while a similar triplet at 7.63 suggests a methylene attached to an sp^2 carbon e.g. methylene group α to the carboxylic function in a fatty acid. The respective integration curve for these two resonances roughly determine the size of the acid between dodecanoic (C12) and stearic (C_{18}). To test this hypothesis the compound was crystallised from methanol saturated with urea in the hope that urea, as it crystallises out from its saturated solution, will carry the contaminating fatty acid or acids with it leaving less of such impurities in solution. This turned out indeed to be the case and repeated

crystallisations of this kind cleared the solution of most of the fatty acid impurity.

Finally the pigment left behind in the solution was crystallised and its NMR spectrum run again. Comparison of this spectrum with that obtained before treatment with urea showed a substantial reduction in the height of the intrusive signal at 8.90 (Fig. 7). The melting point of the purified compound came down to 130 from 168°C, a fall of about 38° showing that the original pigment was an inclusion compound. To obtain further evidence for the correctness of the assumption of formation of an inclusion complex between fatty acids and the compound, the clathrate was reconstituted by crystallising the compound from methanol solution to which authentic palmitic acid had been added. The NMR spectrum of the material obtained through this crystallisation not only reverted to the original pattern but showed a slight enhancement in the height of the intrusive signal since clathrates are seldom perfectly stoichiometric. This left no doubt that the compound initially obtained was a clathrate.

In order to find out the nature of the fatty acids carried away by urea, the urea crystallisate of the first experiment was dissolved in methanol and after methylation with diazomethane was subjected to gas-liquid-chromatography. The resulting graph (Fig. 8) showed that palmitic acid constituted the largest component of the fatty acid impurity which contained besides a little of

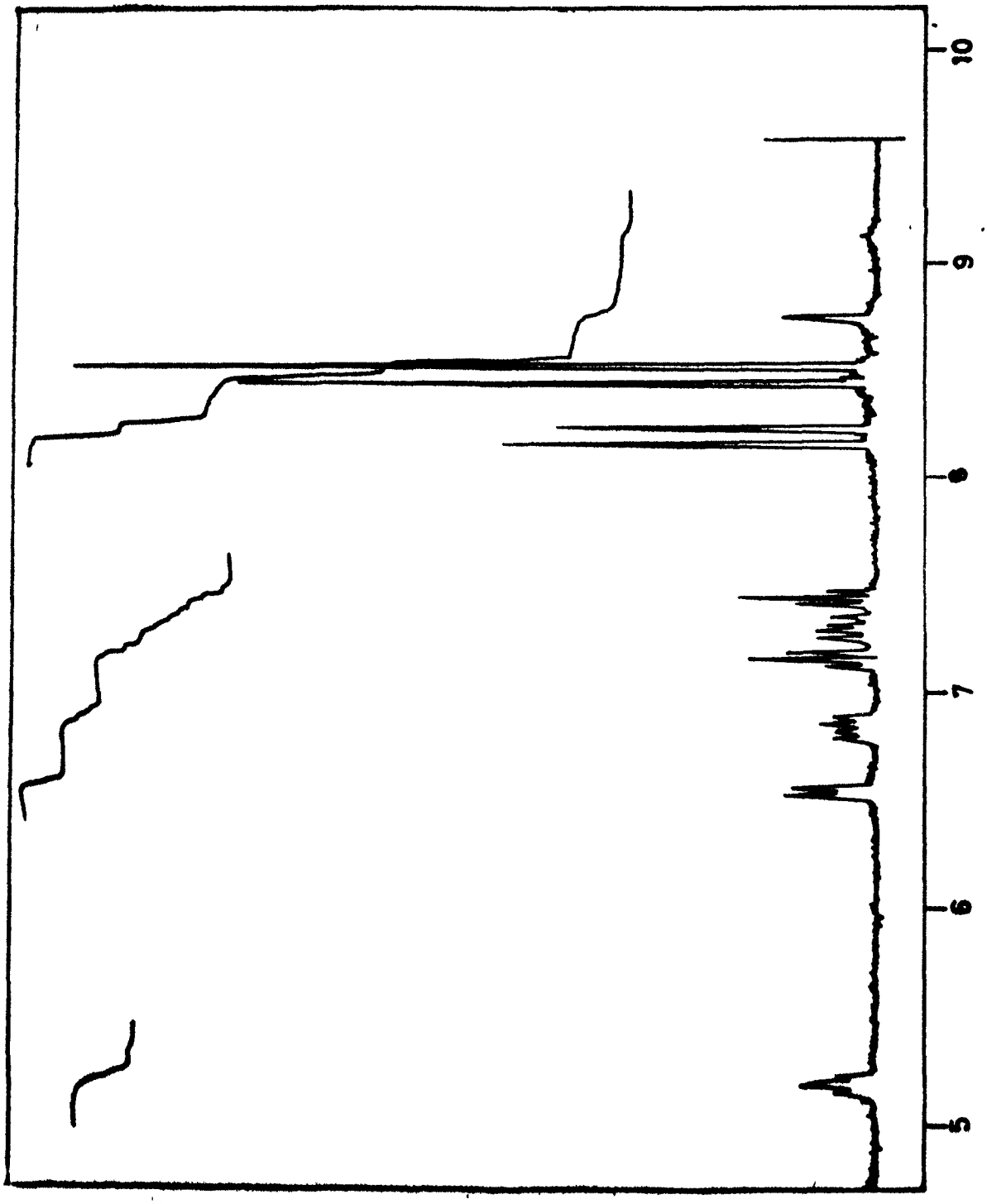


FIGURE- 7

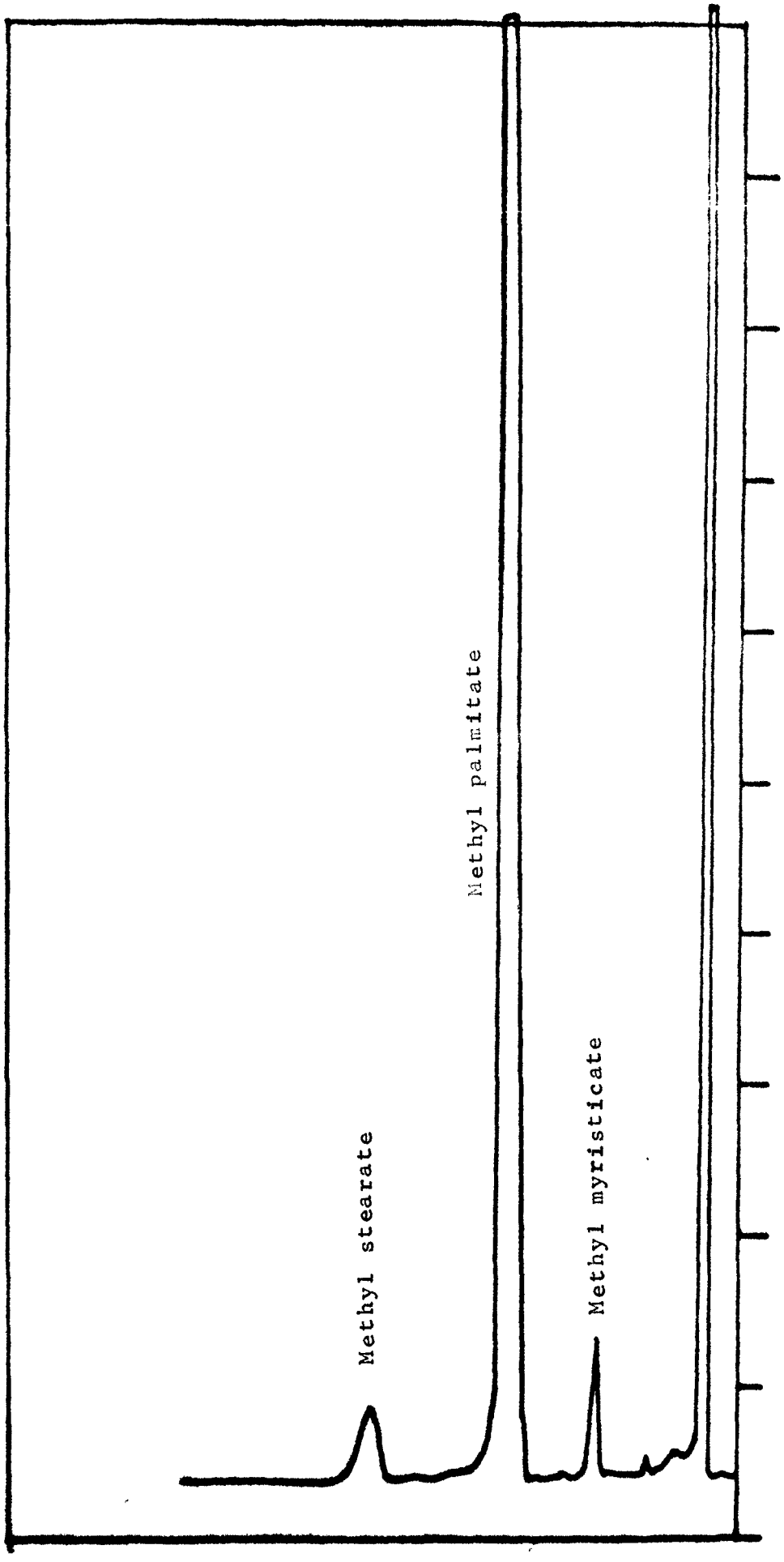
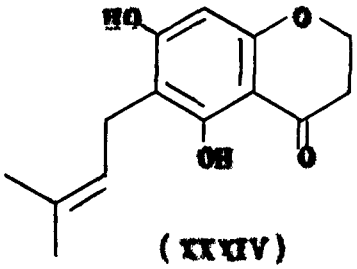


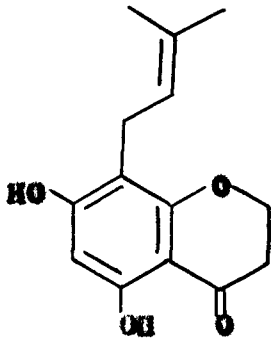
FIGURE- 3

myristic and stearic acids. Relative proton intensities in the NMR spectrum showed the pigment and acid to be in the molar ratio 4:1, as are many similar inclusion compounds between deoxycholeic acid and fatty acids.[74-77]

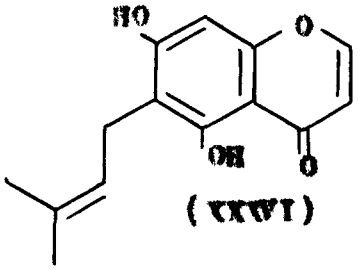
With the molecular formula now firmly established and the nature of the intrusive signal in the NMR spectrum settled, the structural problem was taken up afresh. It was mentioned at the outset that the spectral features are consistent with a chromone or chromanone system and that it contained definitely a γ,γ-dimethylallyl side chain attached directly to the benzene ring and it was also stressed that only one benzylic allylic methylene group is present. With the clarification made above the part structure (i) assigned earlier can be extended to (XXXIV), (XXXV), (XXXVI) and (XXXVII). While it is not possible to easily differentiate between these structures at this stage the singlet of the aromatic proton demands that the remaining positions of the aromatic ring carrying the side chain be substituted and hence the two hydroxyl groups are logically assigned to this ring. The occurrence of such a singlet at 3.68 requires further that it be present in a phloroglucinol type of environment and hence the only ambiguity concerns the position of the γ,γ-dimethylallyl side chain.



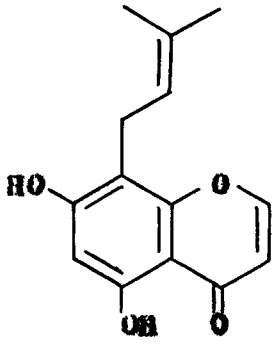
(XXXIV)



(XXXV)

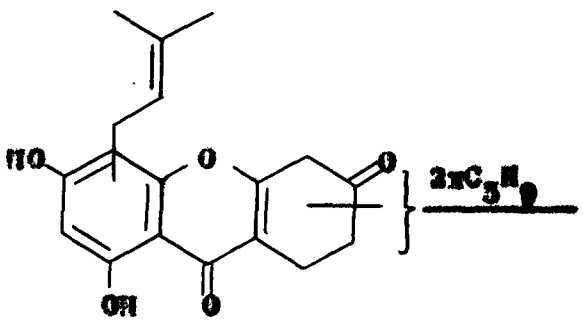


(XXXVI)

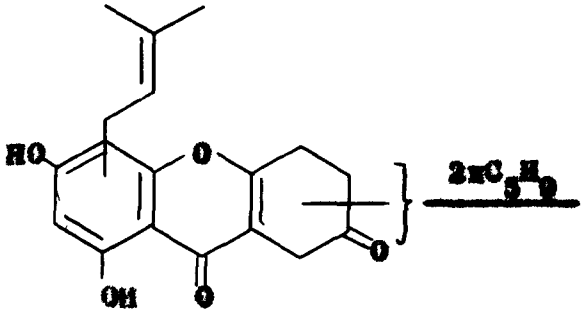


(XXXVII)

The part structures given above have the molecular formula $C_{14}H_{14-18}O_4$ and when this is substracted from $C_{26}H_{34}O_5$, one is left with $C_{14}H_{18-20}O$, substracting C_{10} for the two remaining isoprenoid side chains one is left with four carbon atoms and hence the nucleus of the molecule must be that of hexa or tetrahydroxanthone. As will be seen presently, the number of protons in the midfield region is compatible only with a tetrahydroxanthone, the part structure given earlier thus can be extended to (XXXVIII) and (XXXIX). The carbonyl group can be assigned only to the positions shown since because of its resonance at 1700 cm^{-1} it can not be conjugated with the double bond.

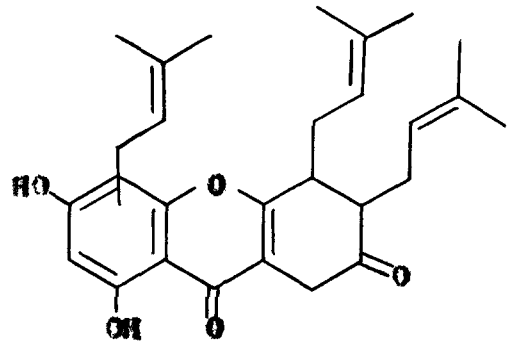


(XXXVIII)

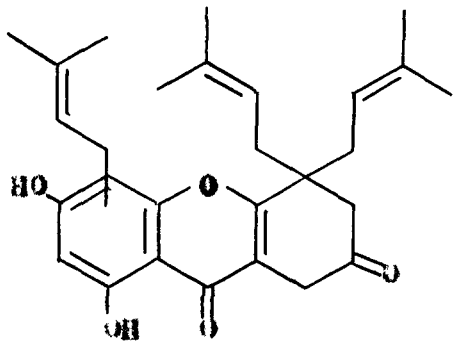


(XXXIX)

The two γ,γ-dimethylallyl groups may be attached to the same carbon atom or their points of attachment to the nucleus may differ but structures such as (XL) are excluded because in the relevant region of the NMR spectrum one does not find any methine multiplet and it is hard to believe that signals of the two methine protons would overlap so as to give rise to a methylene multiplet instead. Apart from this biogenetic considerations also favour structures such as (XLI) in which the two prenyl side chains are attached to the same carbon atom. While it is not possible immediately to assign the multiplets in the NMR spectrum structure (XLI) has the four methylene groups required by the spectrum. Because (XLI) is symmetrical about the plane of the paper it is understandable that signals of the two olefinic protons overlap so as to give rise to the multiplet at 5.15.

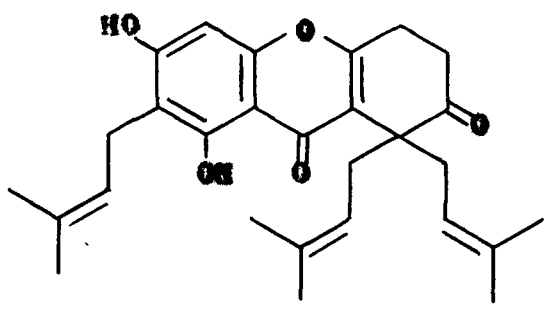


(XL)



(XLI)

While work on this problem had progressed to this stage, Sultanbawa et al[78] published the results of their investigation on Calophyllum parviflorum. They proposed structure (XLII) for the compound, m.p. 137°C, isolated by them and since the melting point is close to that of the purified product obtained from Calophyllum wightianum and the structural features are the same, it seemed likely that the two compounds are identical. Subsequent comparison took time but when carried out showed this to be the case.

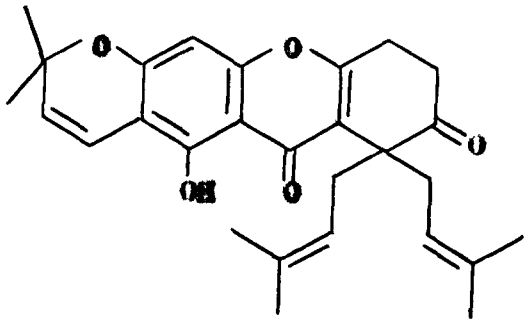


(XLII)

Before turning to a discussion of their conclusions, it is essential to point out that the presence of two symmetrical triplets in the NMR spectrum (7.12 and 7.40 respectively) requires that the ring methylenes be adjacent. This conclusion is naturally based on the assignment of the quartets at 6.84 and 7.30 to the allylic methylenes of the two side chains for reasons to be discussed presently. The disposition of the substituents in the aromatic part of the molecule assumed by them is based on the following observations.

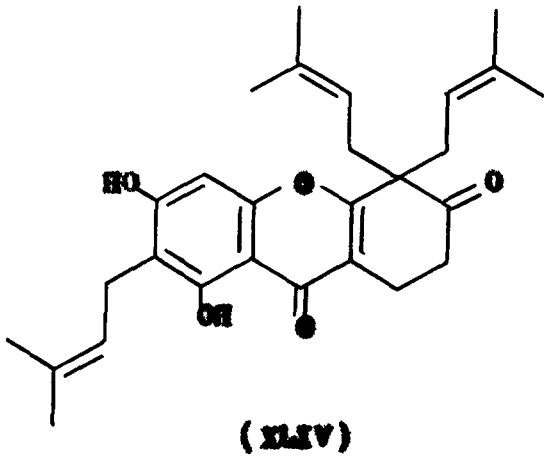
The compound forms a monoacetate at room temperature and a diacetate under more stringent conditions and hence must contain a chelated hydroxy. The side chain is assigned the position shown because the compound gives a positive Gibbs test.[11,12] This coupled with the chemical shift of the aromatic proton establishes that the concerned aromatic ring has phloroglucinol type of substitution and the prenyl side chain does not block the para position. They also carried out a DDQ induced cyclisation to the chromene (XLIII), cyclisation with the non-chelated hydroxyl being assumed because the product still gives positive ferric colour. Since this result was to be expected, the reaction is not really relevant to the structural problem. If it was intended to strengthen the evidence of the Gibbs test, the resulting chromene should have been converted to acetate and the position of the olefinic protons of the chromene examined before and after acetylation. As already pointed out in the discussion, a shift

is only to be expected in the case of linear fusion.[38]



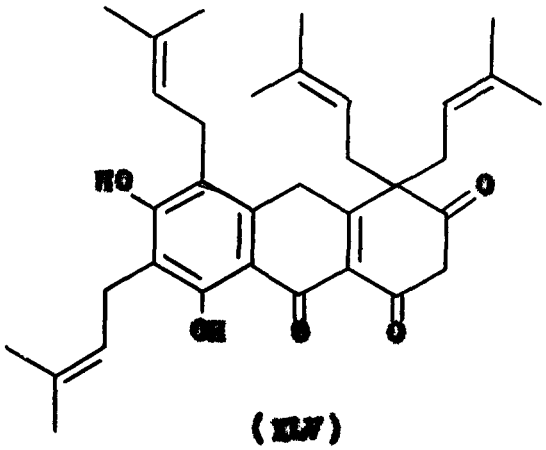
(XLIII)

The authors have given only cursory attention to the non-aromatic part of the molecule and have overlooked that all the data reported by them can be accommodated equally well in the alternate structure (XLIV), the ^{13}C-NMR spectra offer only corroborative evidence for what is already evident from the PMR spectra.



(XLIV)

Tetrahydroxanthones are a comparatively rare group of natural phenolics and to date only a few have been isolated but gambogic acid[36] (XII) and morellin[35] (XI), though more complex structurally, also incorporate tetrahydroxanthone nucleus. In these compounds the orientation of the side chain with respect to the γ-pyrone ring is opposite to that assumed in seyloxanthonone. Harunganin[79] (XLV) though not a xanthone, has again a similar disposition of side chains if the methylene of the central ring is taken to be the equivalent of the ether function. There seems no reason why orientation of the side chains in seyloxanthonone (wightianone) should not be analogous, i.e. the correct structure be (XLIV) rather than (XLII).



(XLV)

The difference between structures (XLIV) and (XLII) involves essentially the position of the carbonyl group since the point of attachment of the prenyl side chains is then fixed

by the NMR spectral requirement that the two ring methylene groups be contiguous. In other words one has to establish whether one is dealing with 6-oxo or 7-oxo tetrahydroxanthone.

As already pointed out, the NMR spectrum of wightianone contains four 2H multiplets. The ring methylenes because of their attachment to sp^3 hybridised carbons and the near planarity of the ring system appear as triplets at 7.12 and 7.40. It is, of course, not possible to say if the triplet at higher field arises from the C-5 methylene and the triplet at lower field from the C-6 methylene or vice-versa in case the structure is assumed to be (XLII). The same argument applies to C-7 and C-8 methylenes if the structure were to be revised to (XLIV). One possible method of distinguishing between these two structures would be to subject the parent unsubstituted tetrahydroxanthone (XLVI) to deuterium exchange under basic conditions. If this experiment were to show that exchange occurs only at the carbon atom conjugated to the carbonyl group (XLVII) then absence of exchange in wightianone would establish structure (XLIV). On the other hand if the methylene peri to the carbonyl group also underwent deuteration (XLVIII) or if partial deuteration occurred at both sites, this approach would not resolve the problem. Tetrahydroxanthone was accordingly synthesised employing the method of Pacquette et al[80] illustrated in scheme 8. It furnished the required compound, as shown by its NMR (Fig. 9), in good yield and this was treated with deuterio-methanol in presence of sodium

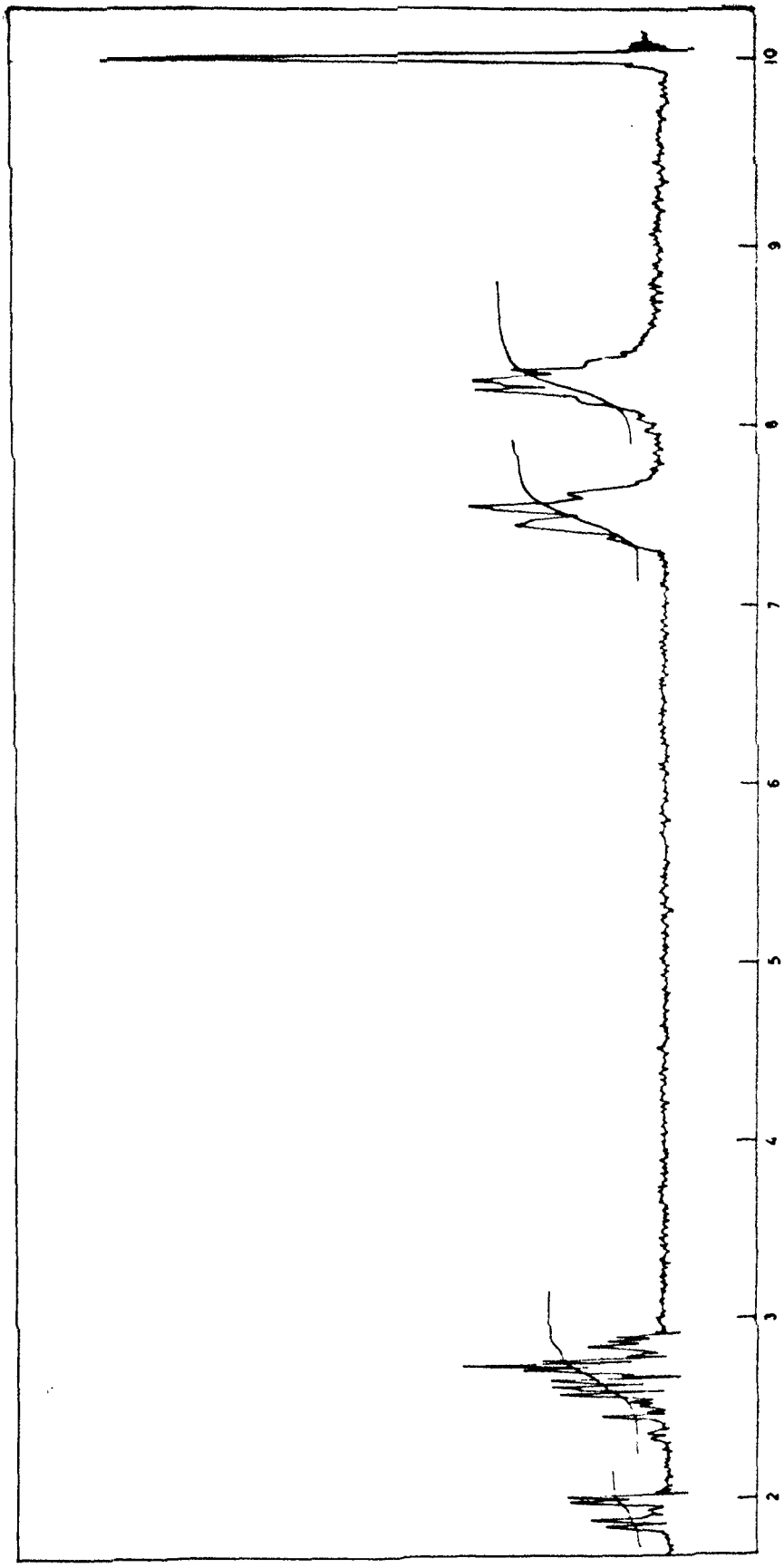
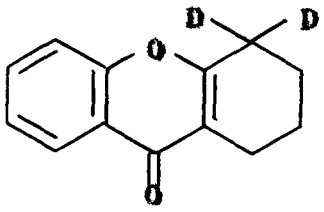
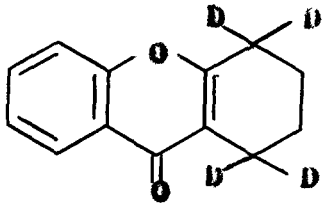


FIGURE- 9

methoxide. When the product was isolated and its NMR run it showed exchange at both sites and accordingly the reaction could not be employed in fixing the orientation of substituents in acyloxanthonone.

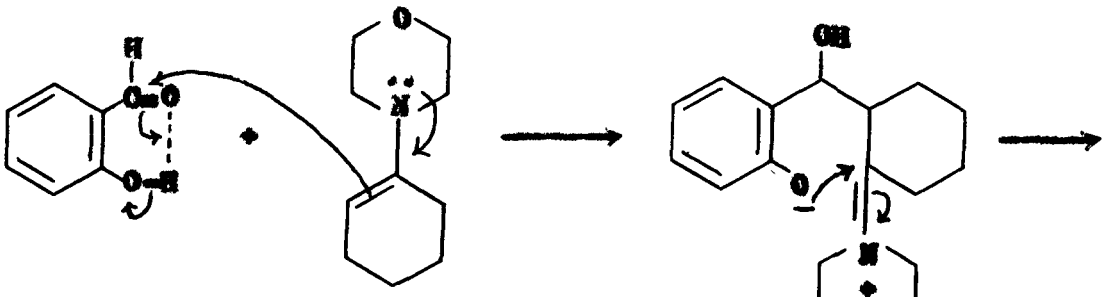


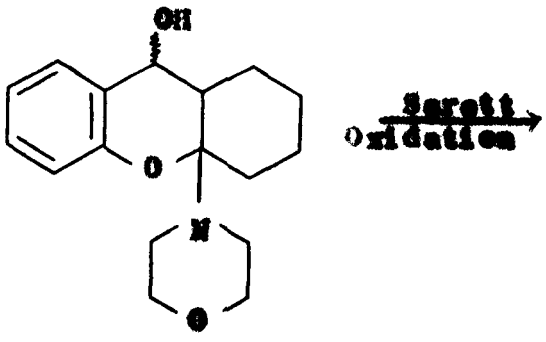
(XLVII)



(XLVIII)

Scheme - 8

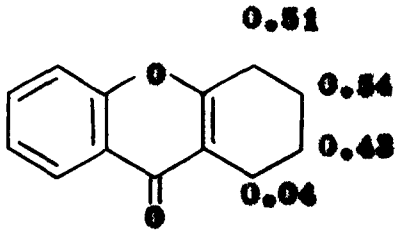




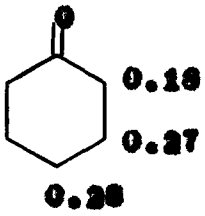
(XLVI)

The orientation of substituents in many heterocyclic aromatic compounds such as coumarins, flavonoids and specially biflavonoids has been settled through application of solvent induced shifts.[31] Such shifts of specific protons of the solute molecules are caused by preferential formation of a sheath of magnetically anisotropic benzene molecules at certain sites of the solute molecules.

The method has not been applied to any large extent in the field of xanthones and specially not in sorting out of a problem of this kind. Basically it involves measurement of benzene induced shifts in tetrahydroxanthone (XLIX) and cyclohexanone (L) on the one hand and in acylxanthonone on the other. In the absence of the six membered ring carbonyl group in acylxanthonone there would have been no need for this kind of data on cyclohexanone. Conclusions based on magnitude of the benzene induced shifts on the positions of the multiplets of the ring methylene groups of acylxanthonone are, of course, valid only if the combination of the benzopyrone system and the carbonyl group towards them are additive. The shifts observed for different protons of tetrahydroxanthone and cyclohexanone are indicated below.

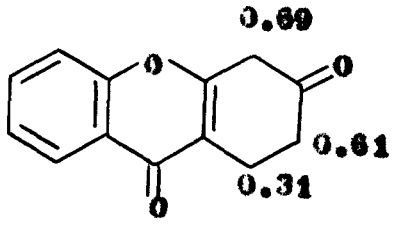


(XLIX)

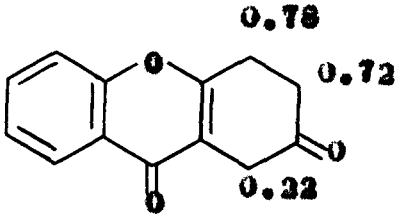


(L)

The calculated shifts for 6 and 7 oxo-tetrahydroxanthones thus can be worked out to values given below (LI and LII respectively).



(LI)



(LII)

The NMR spectra of neoloxanthonone (wightianone) in chloroform and chloroform-benzene (Fig. 10) are reproduced from which the shifts in the position of methylene multiplets can be measured. The observed values turn out to be 0.59 and 0.29. The calculated value for the C-5 and C-6 methylenes in the C-7-oxo compound and C-7, C-8 methylenes in the C-6-oxo compounds are 0.78, 0.72 and 0.61, 0.31 respectively. The values tally almost exactly for the C-6-oxo structure and (XLIV) is thus supported by the evidence of these shifts as well as on biogenetic grounds discussed above.

The mass spectrum of (XLIV) is in complete accord with this structure. It does not, of course, differentiate between (XLII) and (XLIV). The prominent losses from the molecular ion

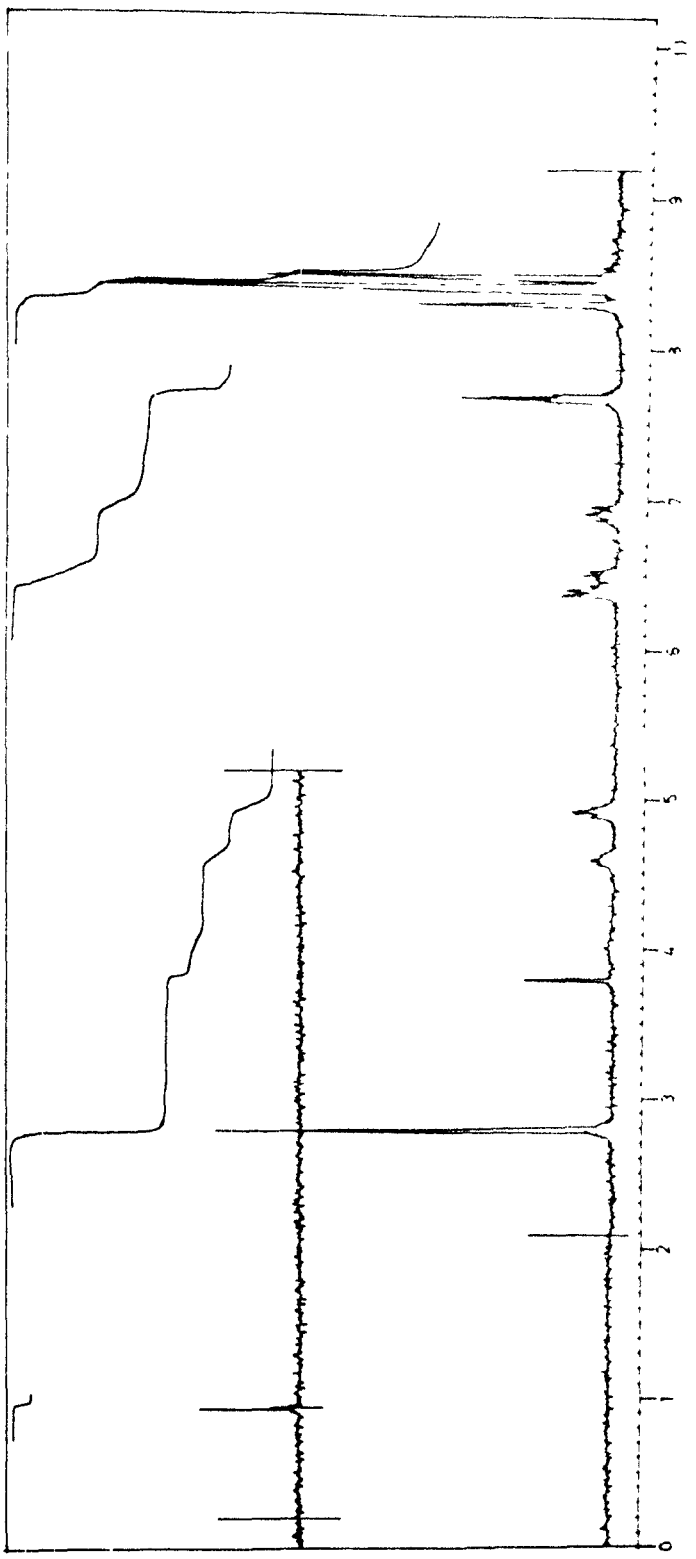
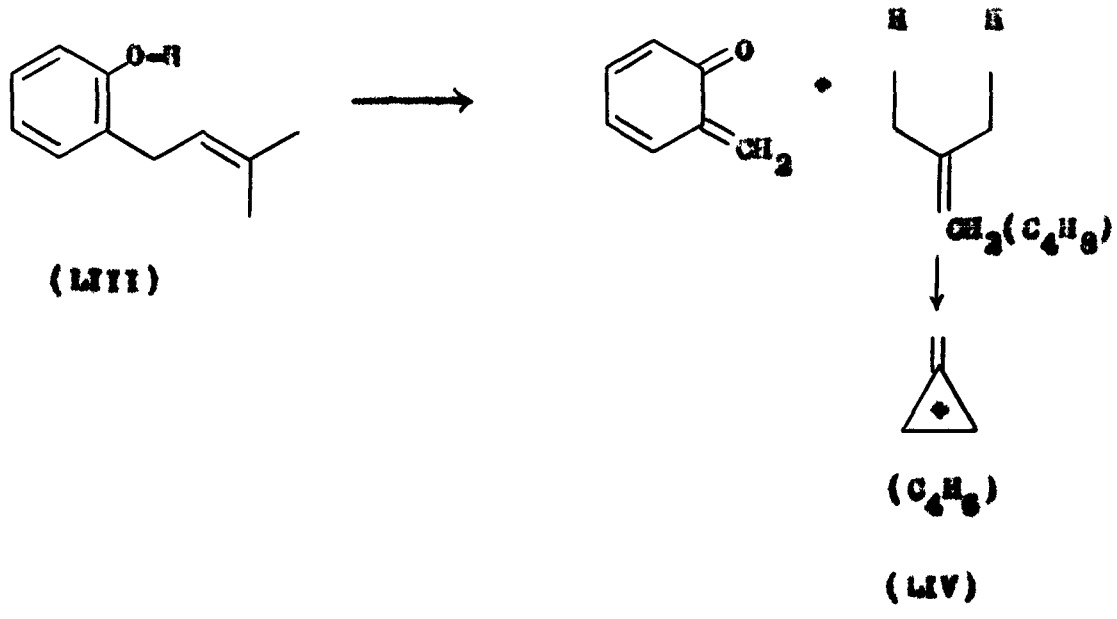


FIGURE- 10

are C_4H_6, C_4H_8, and C_5H_9. The first two are characteristic of structural grouping (LIII) and arise through a McLafferty type hydrogen shift followed by loss of two hydrogens to give a methylene cyclopropyl cation (LIV). The third offers further support for the attachment of remaining two prenyl side chains to the same sp^3 hybridised carbon or placed adjacent to a carbonyl group or both factors are present.[83-84] Another noteworthy feature which was very intriguing at first but becomes clear in the light of subsequent works are the consecutive losses of methylene units appearing in the mass spectrum, these being a characteristic feature of mass spectral fragmentation pattern of fatty acids.

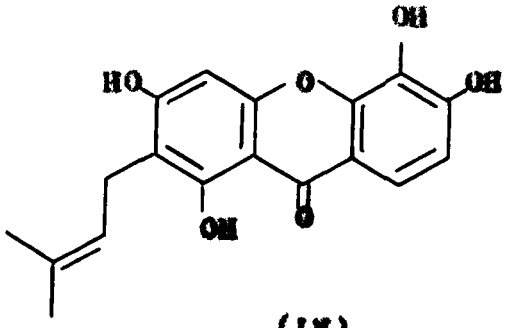


(LIII)

(C_4H_6)

(LIV)

It has previously been suggested[95,96] that the presence of jacareubin (IX) and/or its putative isopreny1 precursor, 2(-3 methyl, 2-butenyl), 1,3,5,6-tetrahydroxyxanthone (LV) may be of taxonomic value in identifying Calophyllum species. Only in the Indian variety of C. inophyllum are these metabolites absent.[97] Our results also demonstrate the unique nature of the Indian varieties of Calophyllum since apart from the xanthone only β-sitosterol and β-amyrin were isolated from Calophyllum wightianum, there being no indication of the presence of jacareubin or its precursor (LV).



(LV)

Tinospora malabarica Miers

Tinospora malabarica (N.O. Menispermaceae) is a climber found extensively in the Malabar region of Kerala in south India and in Goa off the coast of Maharastra. It was collected because of its use in indigenous medicine[91] and because a bitter glycoside, gilloin,[92] had been isolated earlier from Tinospora cordifolia. The petroleum ether extract of the plant contained only hexane soluble constituents of which heptacosanol[93] and β-sitosterol could be identified. The alcohol extract left a sticky mass on evaporation of solvent from which the ethyl acetate soluble material was leached out by repeatedly refluxing with this solvent. The ethyl acetate insoluble portion after repeated chromatography gave a bitter principle which was identified as gilloin, m.p. 228°C. TLC of the ethyl acetate soluble material showed it to be a mixture of several components with marginal differences in R_f values. Repeated chromatography enabled the isolation of two compounds which, from the strong fluorescence in the UV light and colour reactions, appeared to be flavonoid in nature.

Tinosporinone

Tinosporinone, m.p. 182°C, had bands at 1670, 1660, 1605 cm^{-1} in its IR spectrum (Fig. 11) suggesting it to be an aromatic ketone.

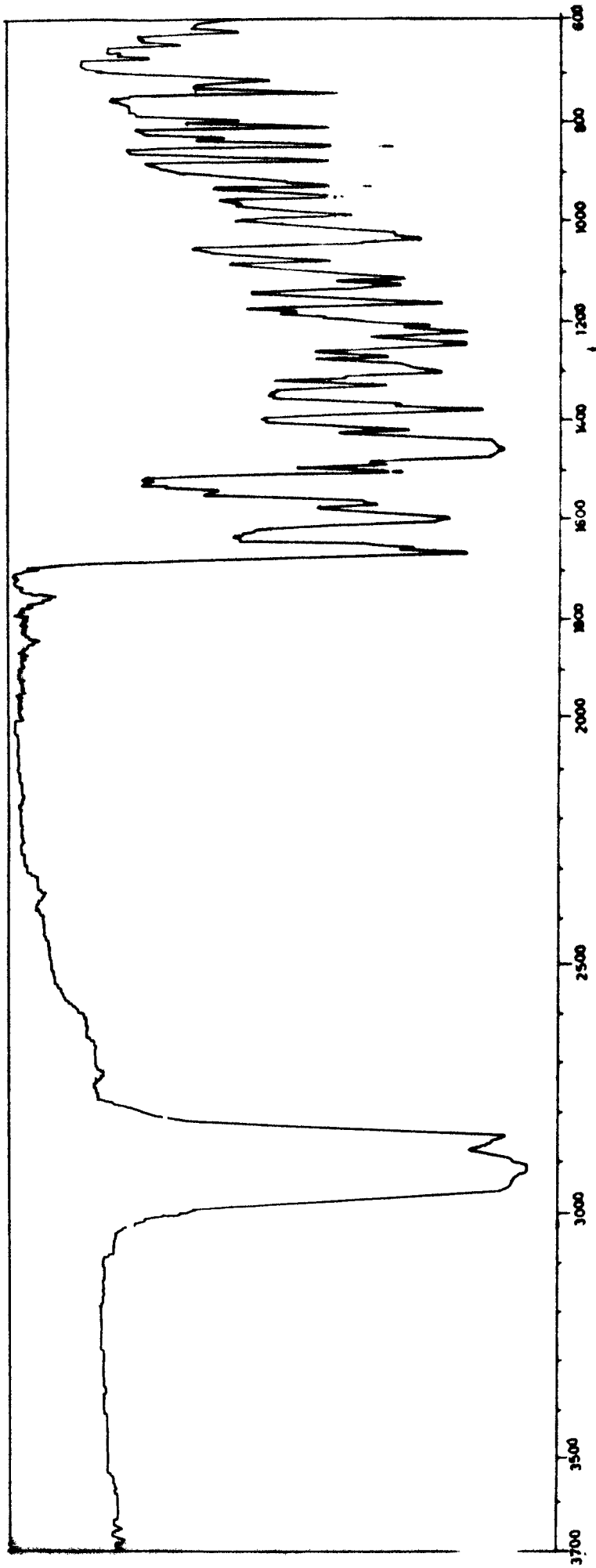


FIGURE- 11

The maxima at 250 and 275 nm in the UV spectrum also point in the same direction but offer no clue as to the nature of the molecule. In the mass spectrum the ion at m/e 311 (100%) was initially mistaken for $M^{+\cdot}$ but since this corresponds to the molecular formula $C_{18}H_{15}O_5$ and a nitrogen atom is not present in the molecule it was considered to arise from the loss of 31 mass units (-OMe) from the molecular ion. The molecular formula $C_{19}H_{18}O_6$ thus arrived at is consistent with the proton count in the NMR spectrum. The NMR spectrum (Fig. 13) shows singlets of methoxy methyls at 6.20, 6.30 and methylenedioxy at 3.95. Since the aromatic region contains signals of six protons there must be two benzene rings in the molecule. The data enumerated thus far is in harmony with the flavonoid structure of the compound but a discordant note is sounded by the presence in the spectrum of a doublet and a quartet at 9.58 and 4.90 respectively. Since these integrate for three and one proton respectively the structure must accommodate a disubstituted ethane moiety, a novel feature of the compound.

Turning to the aromatic region of the spectrum the one proton doublet (J=9 Hz) at 2.01 is obviously due to a proton peri to the carbonyl group. A doublet with the same coupling constant at 3.20 appears to be due to the adjacent proton but acceptance of this assignment forces the assumption that the remaining positions on the benzene ring are blocked which is possible only if the methylenedioxy and one methoxy groups are present in this benzene ring and so the substitution is as shown in the part

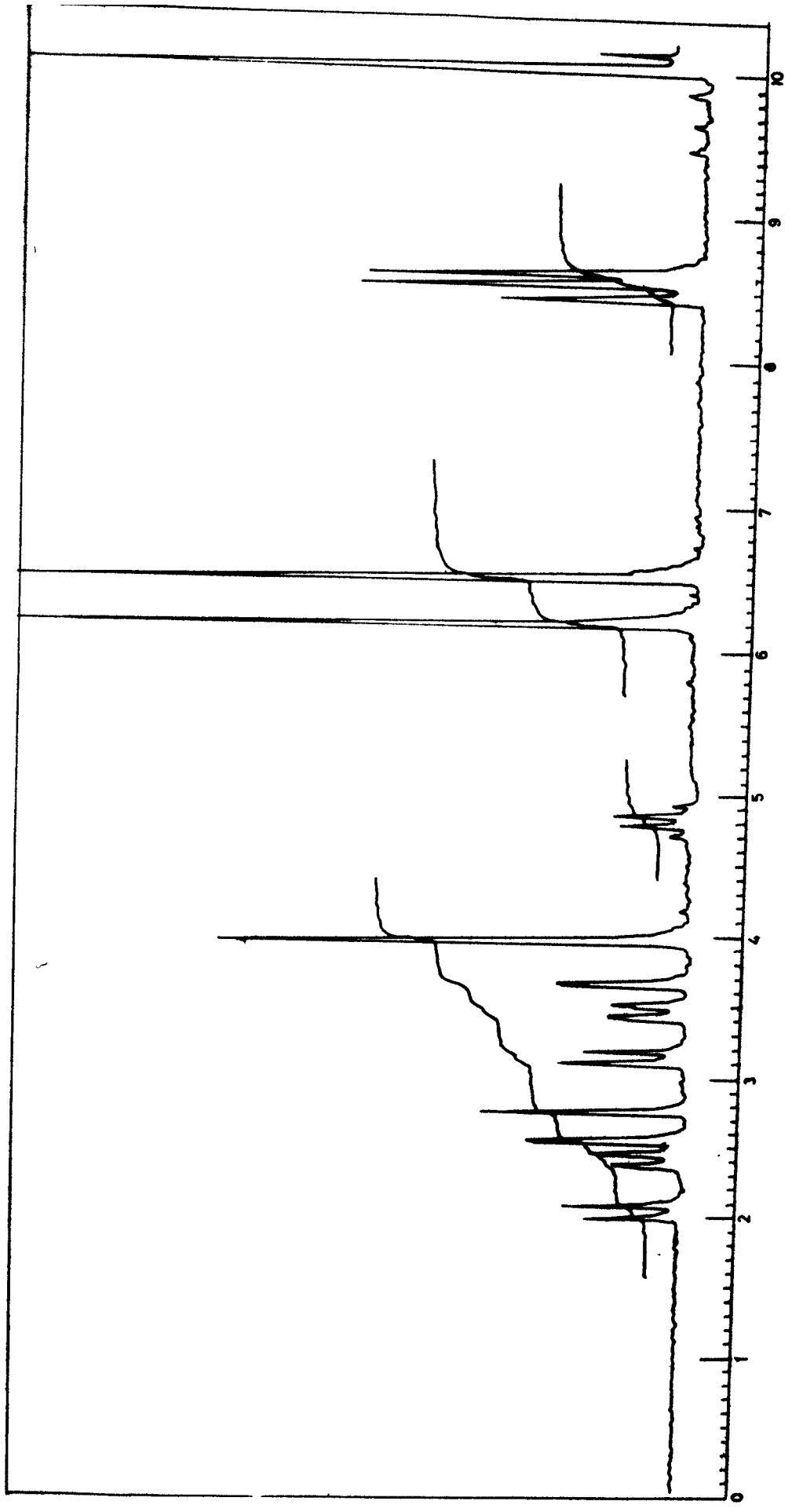
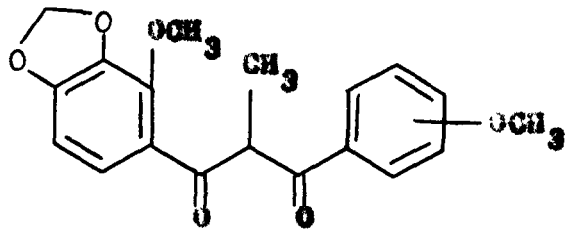


FIGURE- 12

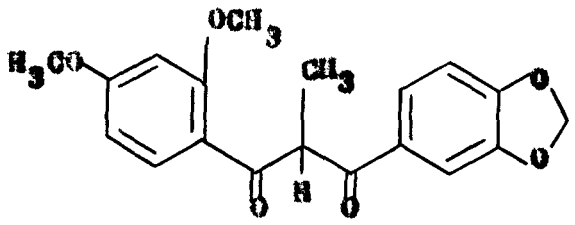
structure (LVI).



(LVI)

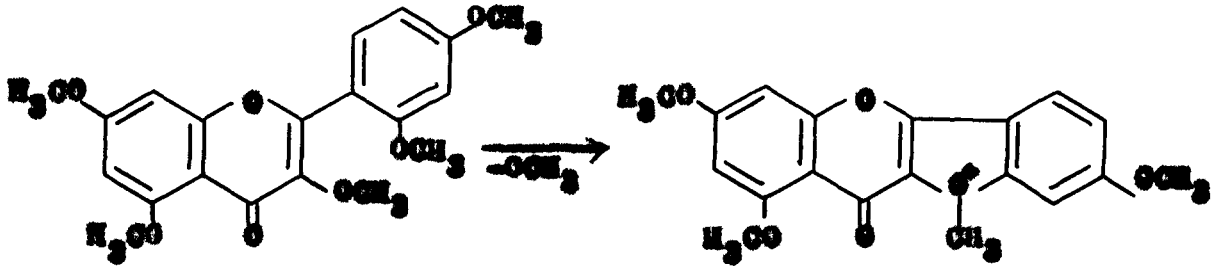
But this leaves only one methoxyl for the second benzene ring, and no matter how this is placed with respect to the point of attachment of the benzene ring to the residual structure the remaining signals in the aromatic region of the spectrum are not explained. The observed coupling pattern is compatible only with the substitution assumed in the structure (LVII) with oxygens equally distributed between the two rings. Thus signals of the three deshielded protons peri to the carbonyl, H-6, H-2 and H-6 appear at 2.05, 2.42 and 2.45 respectively, the arms of the doublet of H-2 being broadened by meta coupling of this proton with H-6. The three signals at the upper edge of the aromatic region of the spectrum are neatly assigned to H-3', H-5' and H-3 from high field to low field. The only unaccounted signal of the spectrum is the singlet at 3.46. Since the rest of the signals integrate for full protons, this can only be due to an impurity

and since the plant is rich in hexane soluble material, a hydrocarbon impurity is suggested.



(LVII)

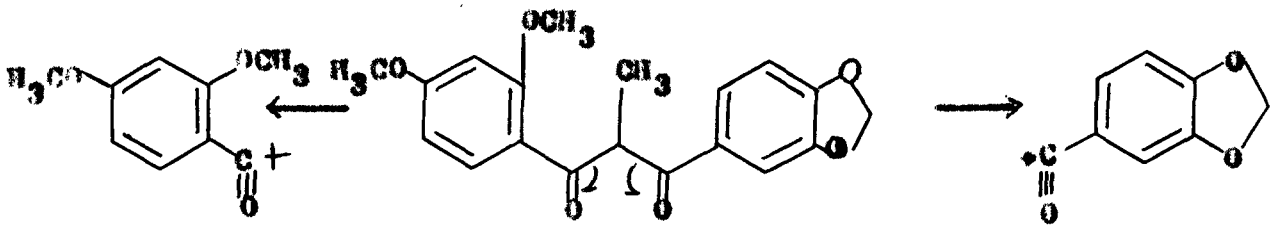
The structure (LVII) is fully supported by the fragmentation pattern observed in the mass spectrum and the absence of the molecular ion peak is also understandable since loss of one methoxyl results in the formation of the resonance stabilised cation (LVIII). Such methoxyl elimination is peculiar to flavones with an -OMe group at C-2' and C-3 and is considered to be of diagnostic value for this type of substitution pattern. Thus in the mass spectrum of 3,5,7, 2',4'-pentamethoxyflavone (LIX) also the $M^{+\cdot}$-OMe peak forms the base peak.[94]



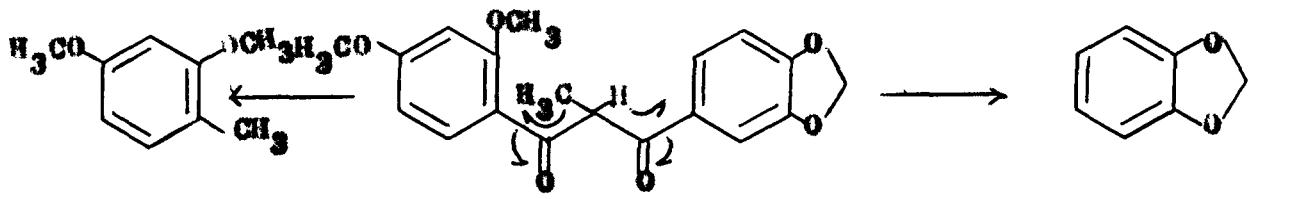
(LIX)

The $M^{\bullet+}$-31 ion in the case of (LVII) can also be derived directly from the enol (LX) by analogy with ortho-OMe cinnamoyl compounds.[95] Other fissions of the molecule on electron impact lead to fragments which can be rationalised as shown in Scheme 9.

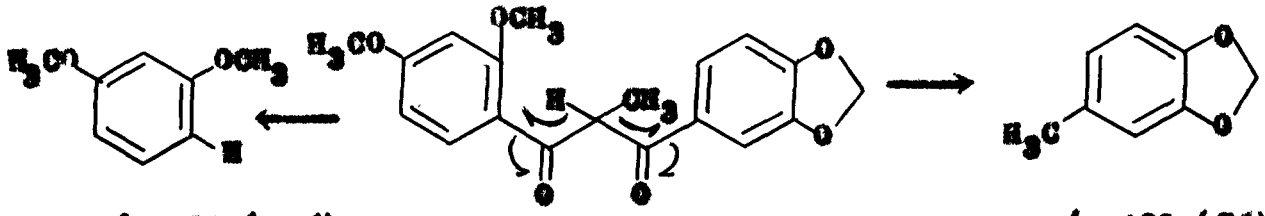
Scheme - 9



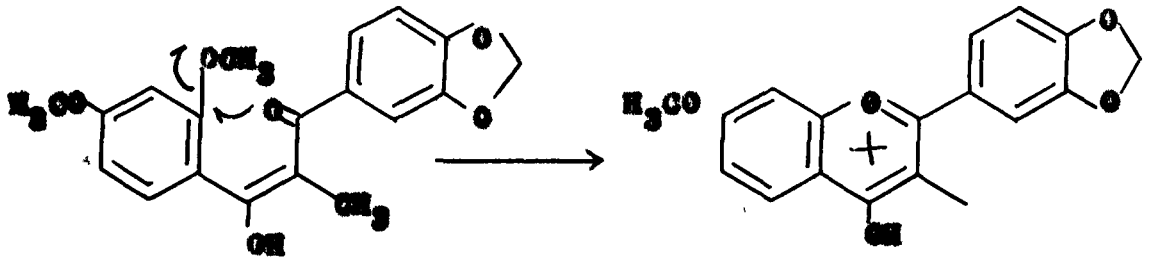
m/e 163 (96%) m/e 149 (40%)



m/e 152 (10%) m/e 122 (15%)

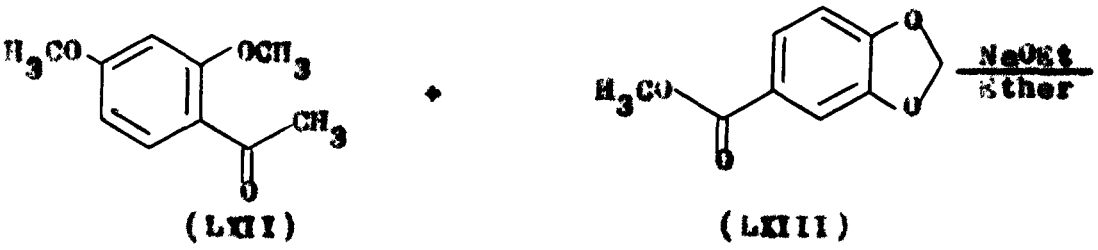


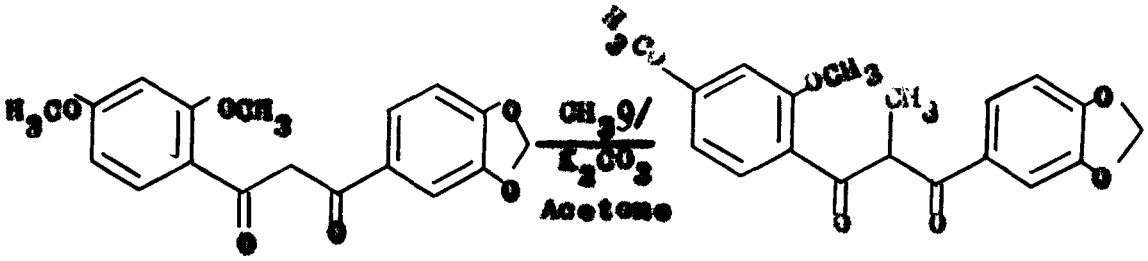
m/e 138 (48%) m/e 136 (7%)



(LX) (LVIII) $M^{\bullet}$ m/e 311 (100%)

Finally the structure (LVII) assigned to tinosporinone was confirmed by comparison with a synthetic sample obtained by C-methylation[96] (CH_3I/K_2CO_3) of milletenone[97] (LXI) which was obtained by condensation of 2,4-dimethoxy acetophenone (LXII) with methyl piperonylate (LXIII).





Comparison of the NMR spectra of tinosporinone with that of dibenzoylmethane e.g. milletenone and ovalitenone[98] shows that it exists entirely in the ketonic form. The same conclusion is to be drawn from the infrared spectrum of the above dibenzoylmethanes as well as those discussed by Wagner et al.[99] The carbony

band is not very pronounced and appears at much lower frequencies. This reluctance to enolisation may be due to steric repulsion that the methyl group would experience in the planar enolic structures and in the non-planar structures the stabilisation afforded by conjugation and hydrogen bonding would be lost. Effects of the kind are perhaps responsible for the fact that methylation of dibenzoylmethane gives only the C-methylation product which is identical, as shown by the NMR spectrum, with the natural sample.

5-Allyloxy, 4',6,7-trimethoxy Flavone

The second product, m.p. 162°C, which could be obtained from the ethyl acetate soluble fraction of the alcohol extract showed $M^{+\cdot}$ at m/e 368. The molecular formula on this basis works out to $C_{21}H_{20}O_6$. The IR spectrum (Fig. 13) of the compound shows a carbonyl band at 1660 and the UV has maxima at 270, 320 nm. Coupled with the positive Shinoda's test[100] the data suggests a flavone nucleus for the compound and its NMR spectrum (Fig. 14) confirms this through presence of the singlet of the C-3 hydrogen at 3.45. The NMR spectrum shows three methoxy methyls at 6.05, 6.10 and 6.15, a 2H multiplet at 4.70 and an octet integrating for one proton at 3.80. The methylene and methine multiplets at this value can arise through an O-allyl group presence of which was indicated at the outset by the peak at $M^{+\cdot}$-O-allyl at m/e 311.

With the flavone nucleus settled, the location of the substituents has to be established to complete the structure. The task is simplified in this case by the presence of 2H doublets at 3.90 and 4.05 (J=8, 2H) constituting an A_2B_2 system and therefore, establishing an oxygen function at C-4' only in ring B. The singlet at 3.85 can be allotted either to the C-8 hydrogen in 5,6,7 substituted flavone (LXIV) or to the C-6 hydrogen in 5,7,8 substituted compound (LXV).

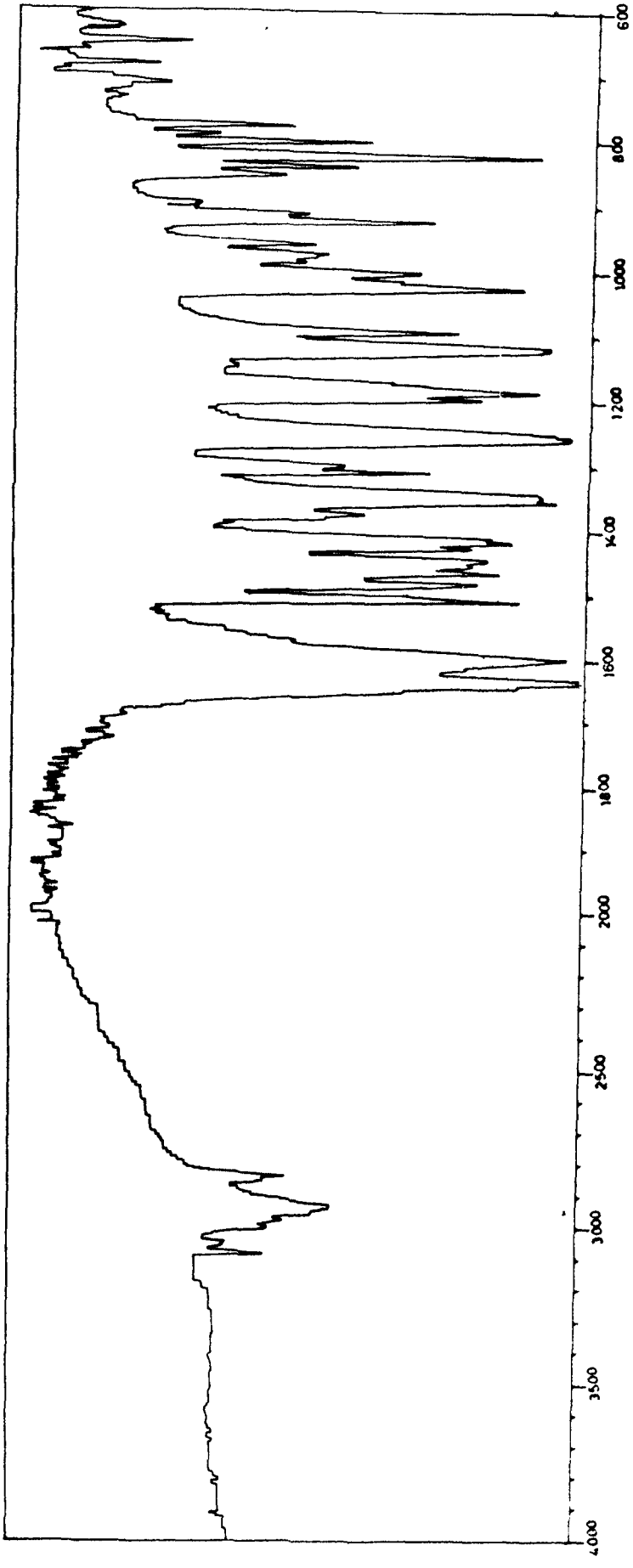


FIGURE- 13

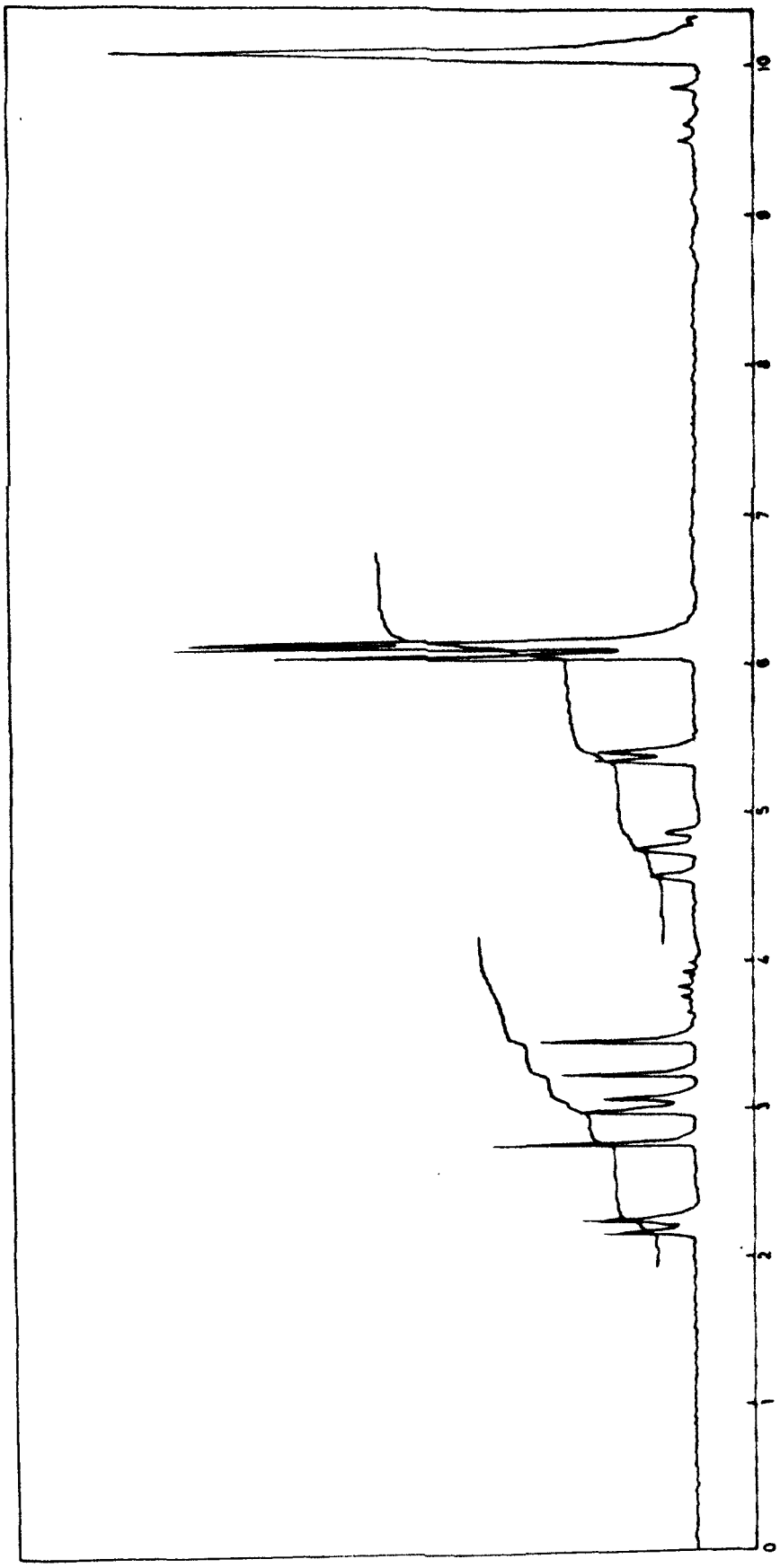
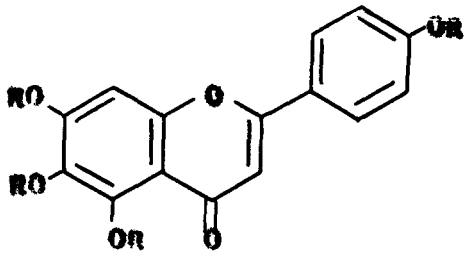
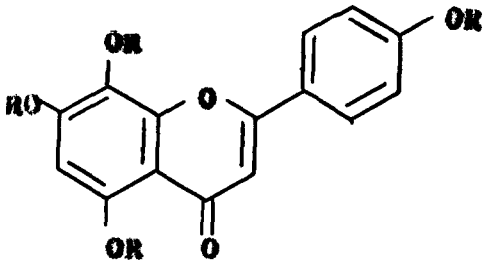


FIGURE- 14



(LXIV)



(LXV)

The choice between these two alternatives would have been difficult had all the three oxygens in this ring carried methyls but the allylic side chain simplifies the task and indeed makes it possible to reach a definite conclusion regarding the substitution in this ring.

Under the conditions of Claisen rearrangement[101] the allyl side chain was found to have migrated and the product (LXVI) gives a strong ferric reaction and shows evidence of chelated hydroxyl in its NMR spectrum (Fig. 15). The allylic side chain must therefore be attached to C-6 oxygen. Since a dihydrobenzofuran is not a by-product of the reaction[102] and is also not obtained on treatment with acids, the side chain must have migrated to the C-6 and not to the C-8 position, thus conclusively establishing the 5,6,7 oxygenation pattern in the molecule (LXVII).

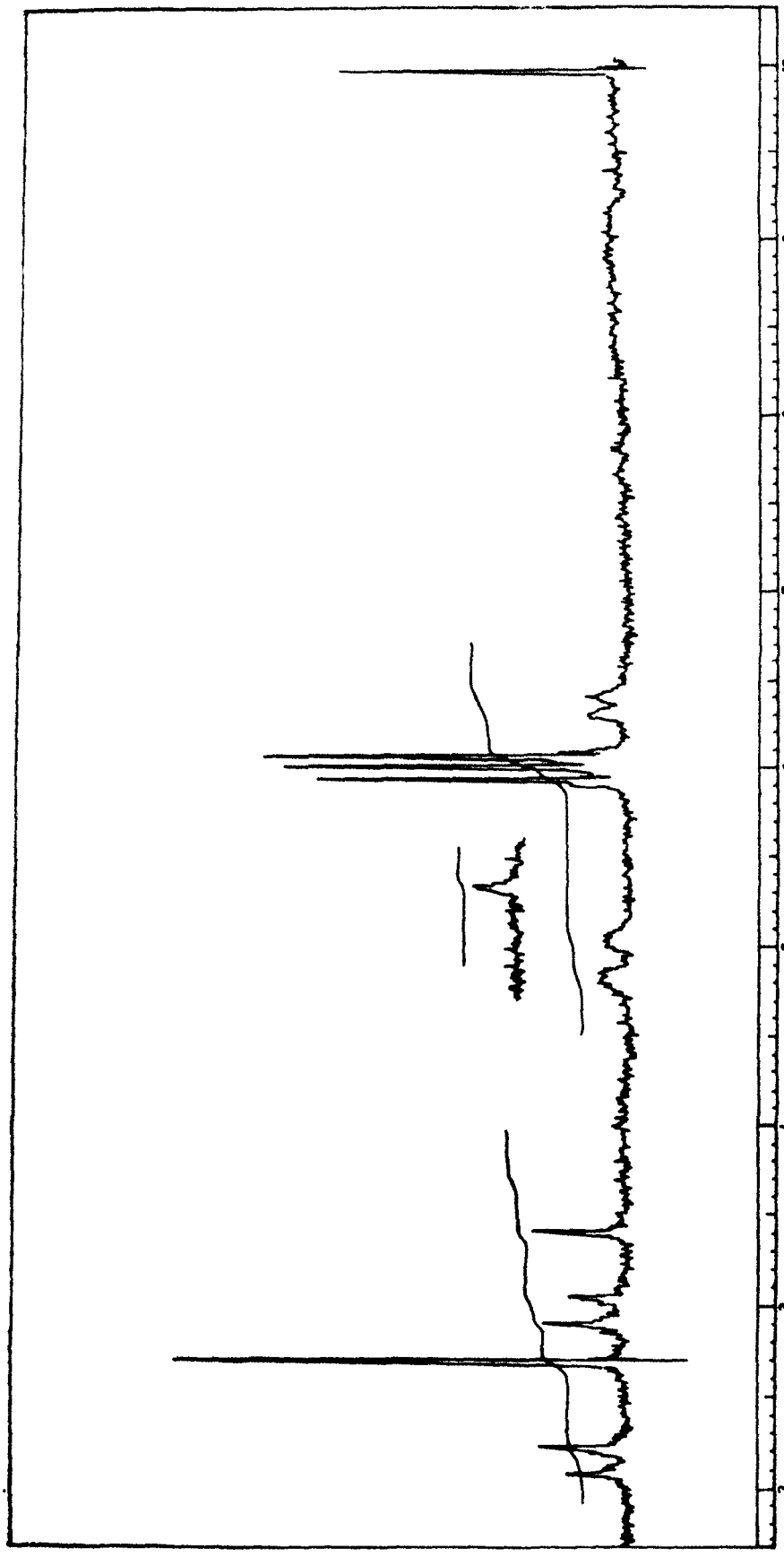
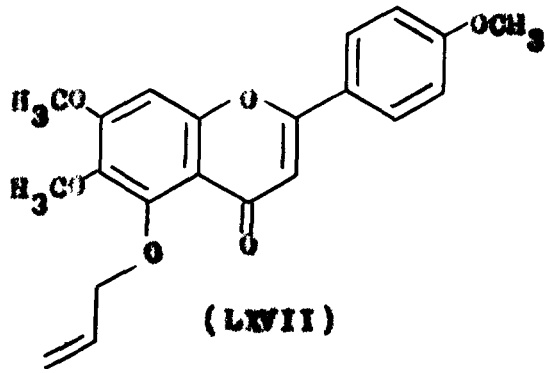
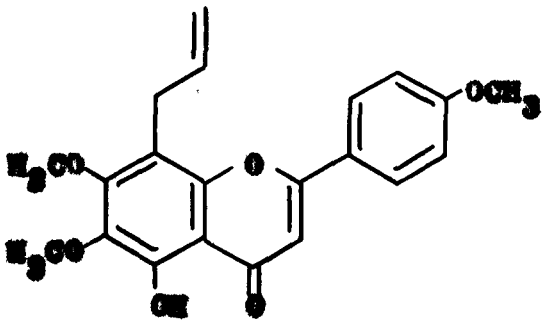


FIGURE- 15

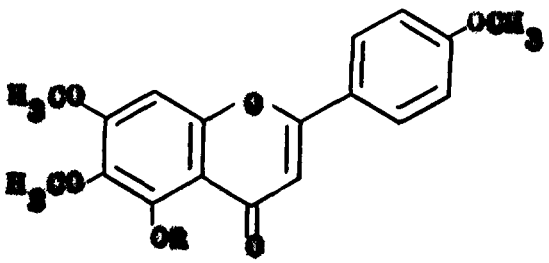


(LXVII)

Finally, mild acid hydrolysis eliminates the allylic side chain to form a product (LXVIII) which also gives strong ferric reaction and offers evidence of chelated hydroxyl in its NMR spectrum (Fig. 16). The melting point, 190°C, of the hydrolysis product agrees exactly with that for the known 4',6,7-trimethoxy, 5-hydroxyflavone.[103] The much higher melting point of 4',7,8-trimethoxy, 5-hydroxyflavone,[104] 218°C, leaves no possibility of confusion. This was further corroborated by the melting point of the acetate (LXIX) which also agrees with the 5,6,7 substitution and the large paramagnetic shift (Fig. 17) of the singlet also point to a free para position.[105]



(LXVI)



(LXVIII) R=H
(LXIX) R=Ac

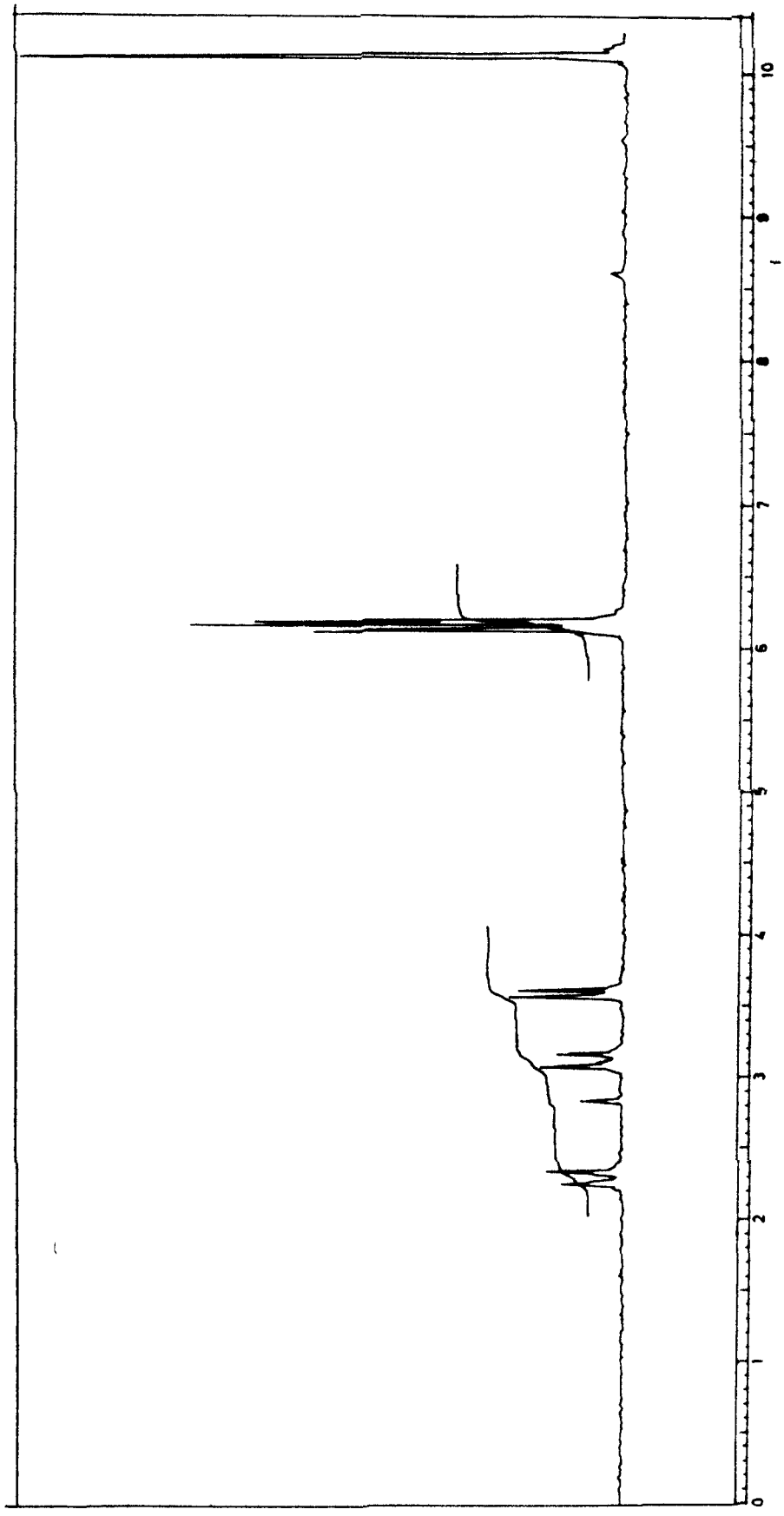


FIGURE- 16

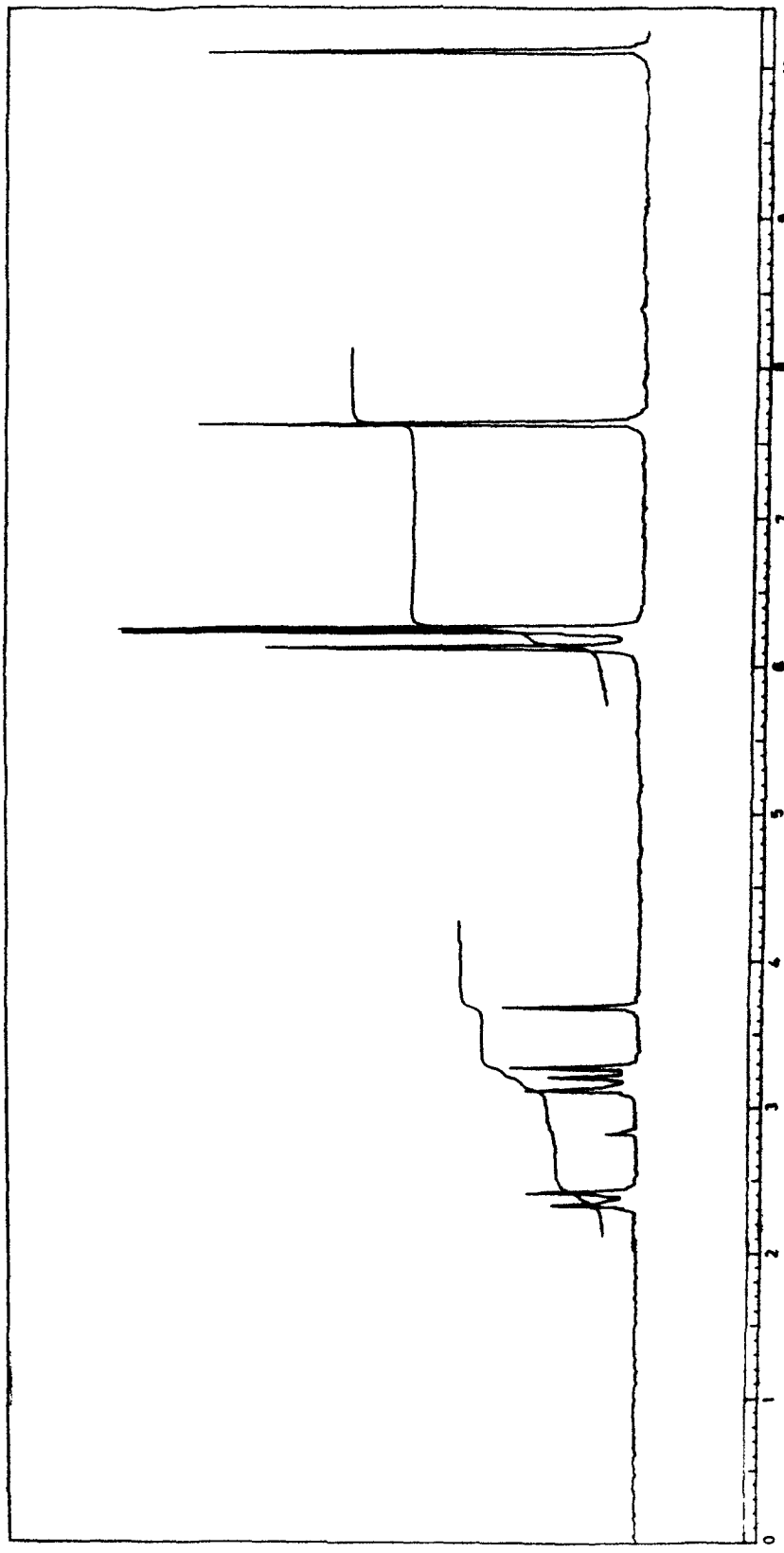
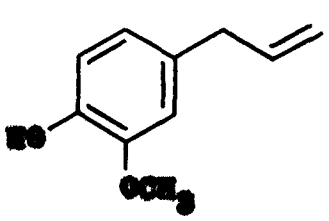


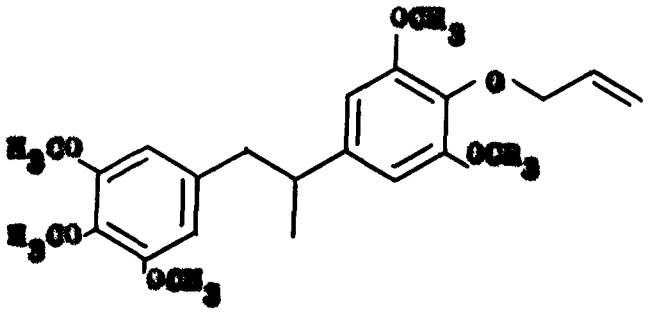
FIGURE- 17

The mass spectrum of (LXVII) has all the fragments expected of a flavone with the indicated substitution. Thus it shows prominent peaks through the losses of methyl and allyl groups. The main fragments indicated by the mass spectrum can be rationalised on the basis of the scheme shown (Scheme 10).

While structurally the compound is simple, it is novel in that it is the first allyloxy flavone isolated. Allyloxy compounds in nature are rare as against the α,α and γ,γ-dimethylallyloxy compounds which are of common occurrence. The allylic side chain in aromatic compounds such as eugenol (LXX) is supposed to be derived from cinnamic acid. Initially it was thought that the carboxylic group of cinnamic acid is reduced to methyl but in a recent paper Canonica *et al*[106] report that the carboxylic group is completely oxidised and the residual side chain is then methylated. For the allyloxy side chain it has been suggested that it arises through retro-Claisen rearrangement from C-allyl compounds. The Claisen rearrangement may be combined with a Cope rearrangement step which is believed to be the process involved in the biogenesis of aureln (LXXI).[107]

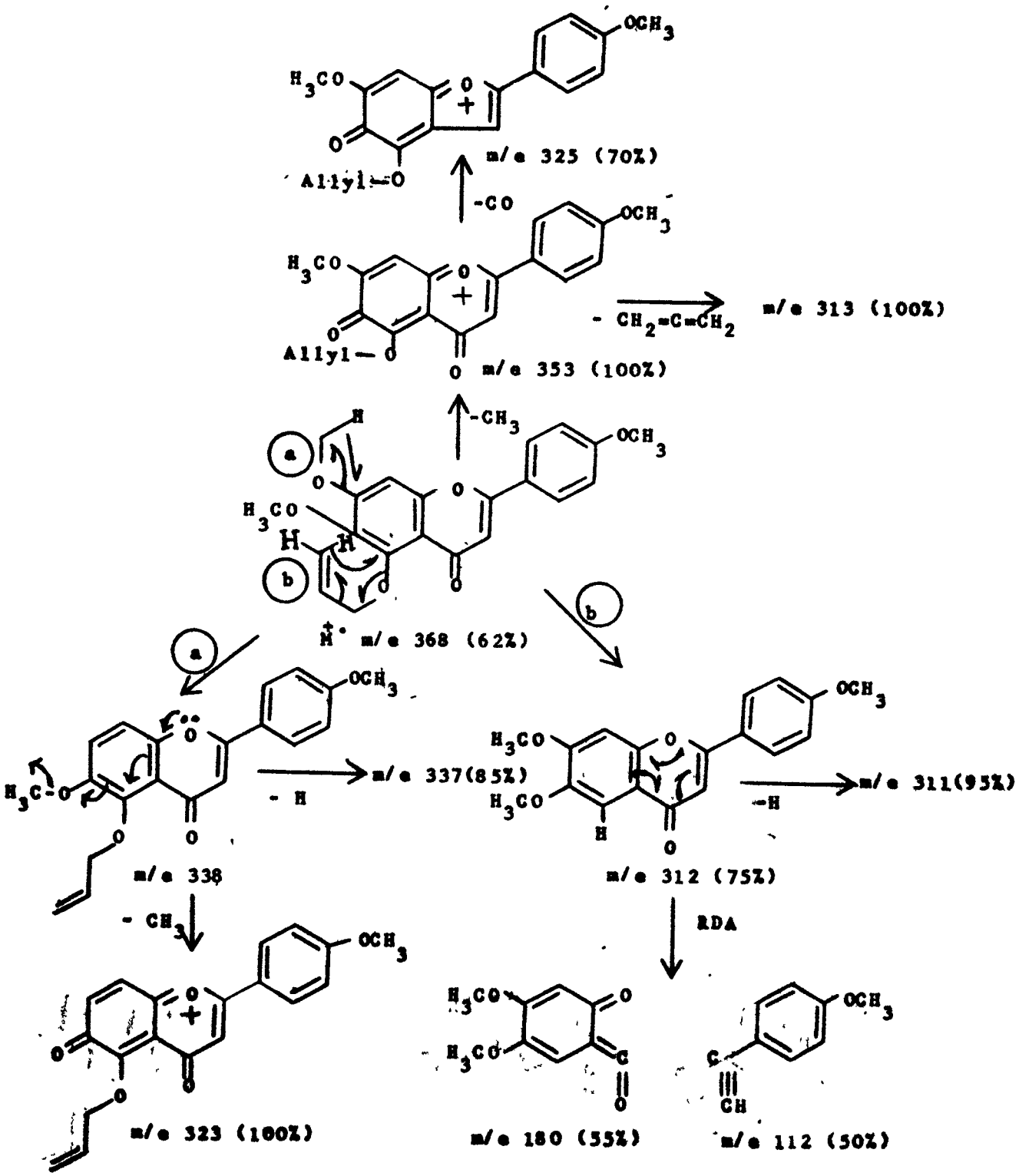


(LXX)



(LXXI)

SCHEME- 10


m/e 325 (70%)
-CO
- CH2=C=CH2
m/e 313 (100%)
m/e 353 (100%)
-CH3
m/e 368 (62%)
- H
m/e 337(85%)
-H
m/e 311(95%)
m/e 338
m/e 312 (75%)
- CH3
RDA
m/e 323 (100%)
m/e 180 (55%)
m/e 112 (50%)

Verbesina encelioides Benth

Verbesina encelioides, a herbaceous plant resembling sunflower (Family-Compositae) is widely distributed in the gangetic plain. It is rich, like other composites, in essential oils which are the subject of a recent paper by Bohlmann et al in which a number of eudesmane derivatives are reported.[108] In work carried out about the same time Tewari et al isolated pseudo-taraxasterol and its derivatives.[109] A number of flavone C-glycosides have also been isolated from Verbesina encelioides.[110]

Verbesina encelioides had been collected before these reports were available and since removal of solvent from its petroleum ether extract left a large amount of residue it appeared of interest to check at least the hexane insoluble portion of the extract for the presence of other constituents. This led to the isolation of two compounds the relationship of which to each other as alcohol and acetate was apparent from the IR and NMR spectra and confirmed chemically. The m.p. of the alcohol and the acetate differed substantially from those reported for pseudo-taraxasterol and its acetate with the former melting at 196°C i.e. 22°C lower and the latter at 208°C i.e. 26°C lower. The alcohol, however, gave the diagnostic colour reaction of triterpenes and its NMR had the characteristic contours of the triterpene nucleus at higher field.

Triterpene melting points have been found to differ frequently due to dimorphism e.g. oleanolic acid acetate crystallised from benzene melts at 197-98 whereas that crystallised from methanol melts at 257-58°C.[111] The substantial difference in melting points could, therefore, be misleading and Dr. H.P. Tewari was approached for a sample of pseudo-taraxasterol so that a direct comparison could be made. Since no reply was received it was decided to do the necessary structural work on the compound. As a class triterpenes have proved to be most amenable to mass spectroscopy which can be said to have replaced earlier degradative methods and, combined with NMR, has circumvented the need for selenium dehydrogenation. The conclusions drawn here also rely mainly on the evidence of these two spectra as the UV spectrum of the triterpene alcohol established merely the absence of conjugation and in the infrared region the only band of interest was of -OH stretching at 3300 cm^{-1} (Fig. 18). As for the number of OH groups the solubility in all organic solvents except methanol, comparatively low melting point for triterpenes and considerable mobility on TLC plates all suggested that only one or two hydroxylic functions were present. This was confirmed through acetylation which supplied the same compound as that isolated from the extract.

The NMR spectrum of the alcohol (Fig. 19) shows a number of methyls at higher field and three multiplets at 6.75, 5.40 and 4.78 of which the one at 5.40 appears to be due to an impurity.

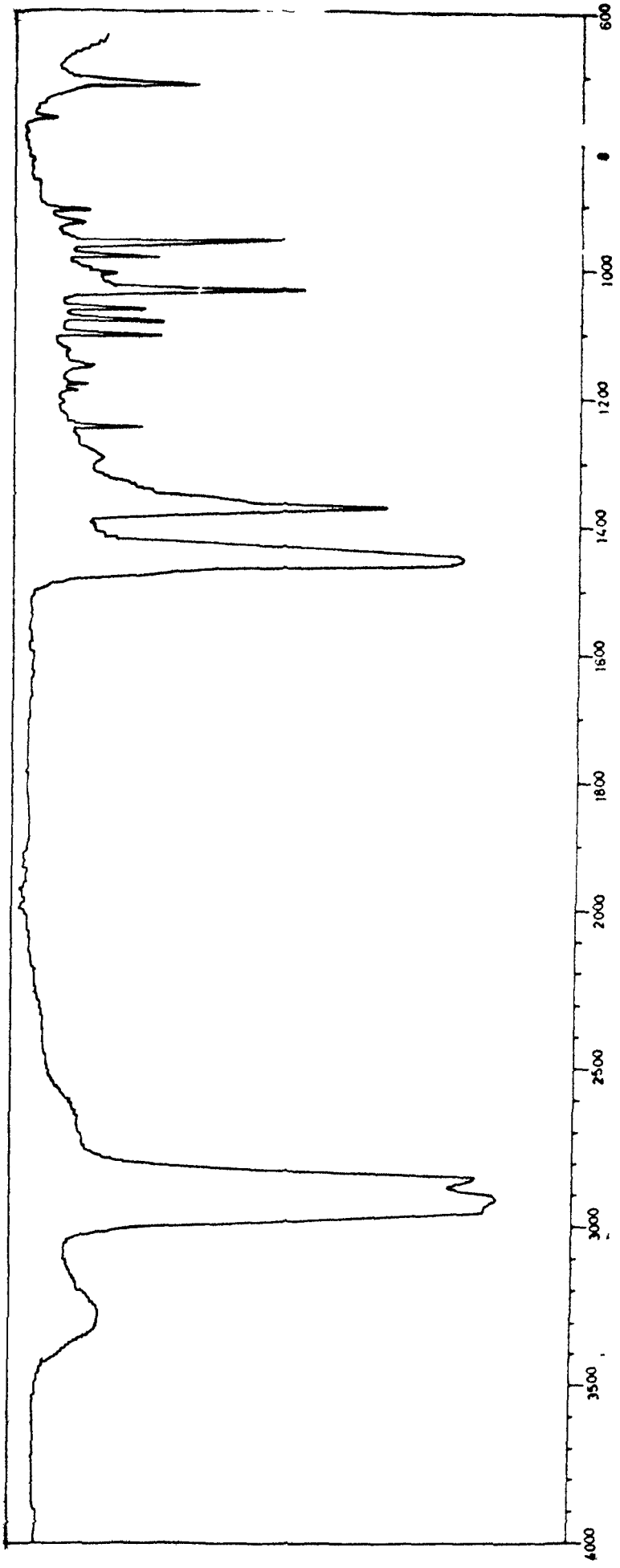


FIGURE- 18

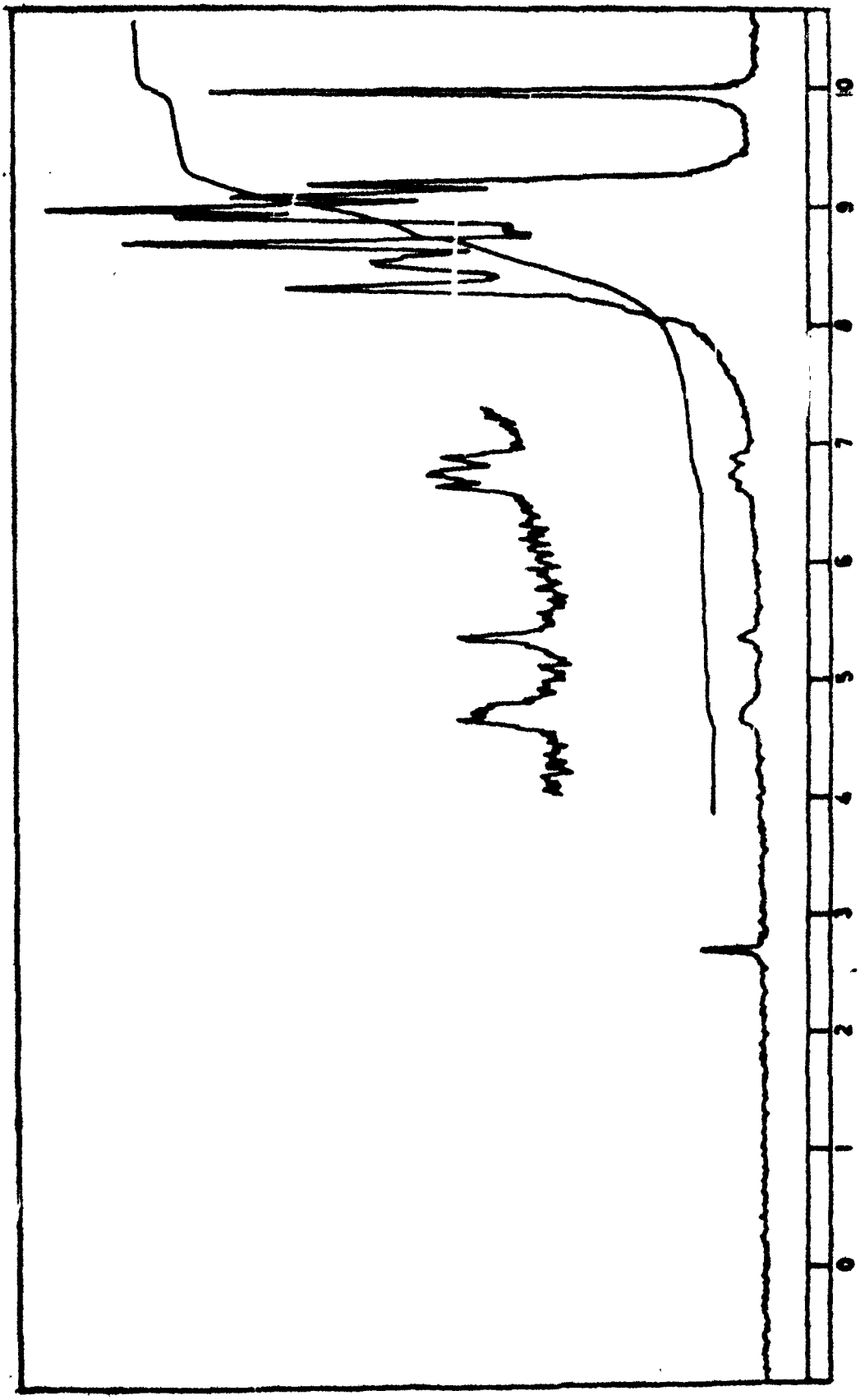


FIGURE- 19

This is apparent from the integration curve for the rise of the integral over the multiplet at higher field is three units and over the multiplet at 4.75 two units whereas over the signal of the impurity the rise is so small as not to be readable since the integral amplitude was adjusted to keep it on scale at higher field where resonances of the methyl and methylene groups occur. The rise of the integral over the multiplets in the centre of the spectrum is too small but a clearer picture emerges from inspection of the higher amplitude recording of the multiplets. Comparison of the height and breadth of these multiplets can only lead to the conclusion that if the signal at 4.75 is of one proton the adjacent signal can be only for one fourth proton. The some what larger area under the multiplet at 6.75 might be due to carry over of the signal of the methylene protons. The conclusion is corroborated further in the spectra of compounds resulting through attempted hydrogenation to be discussed presently.

In the spectrum of the acetate (Fig. 20) the signal at 6.75 is found shifted to 5.40. This characterises the multiplet at 6.75 in the alcohol spectrum as that of a $\underline{CH}$-OH proton paramagnetic shifts of this magnitude being quite normal on acetylation. The position of the multiplet at 4.70 does not change. The impurity present in the alcohol also contaminates the acetate and is responsible for the distortion of the symmetrical triplet of the proton under the acetate oxygen.

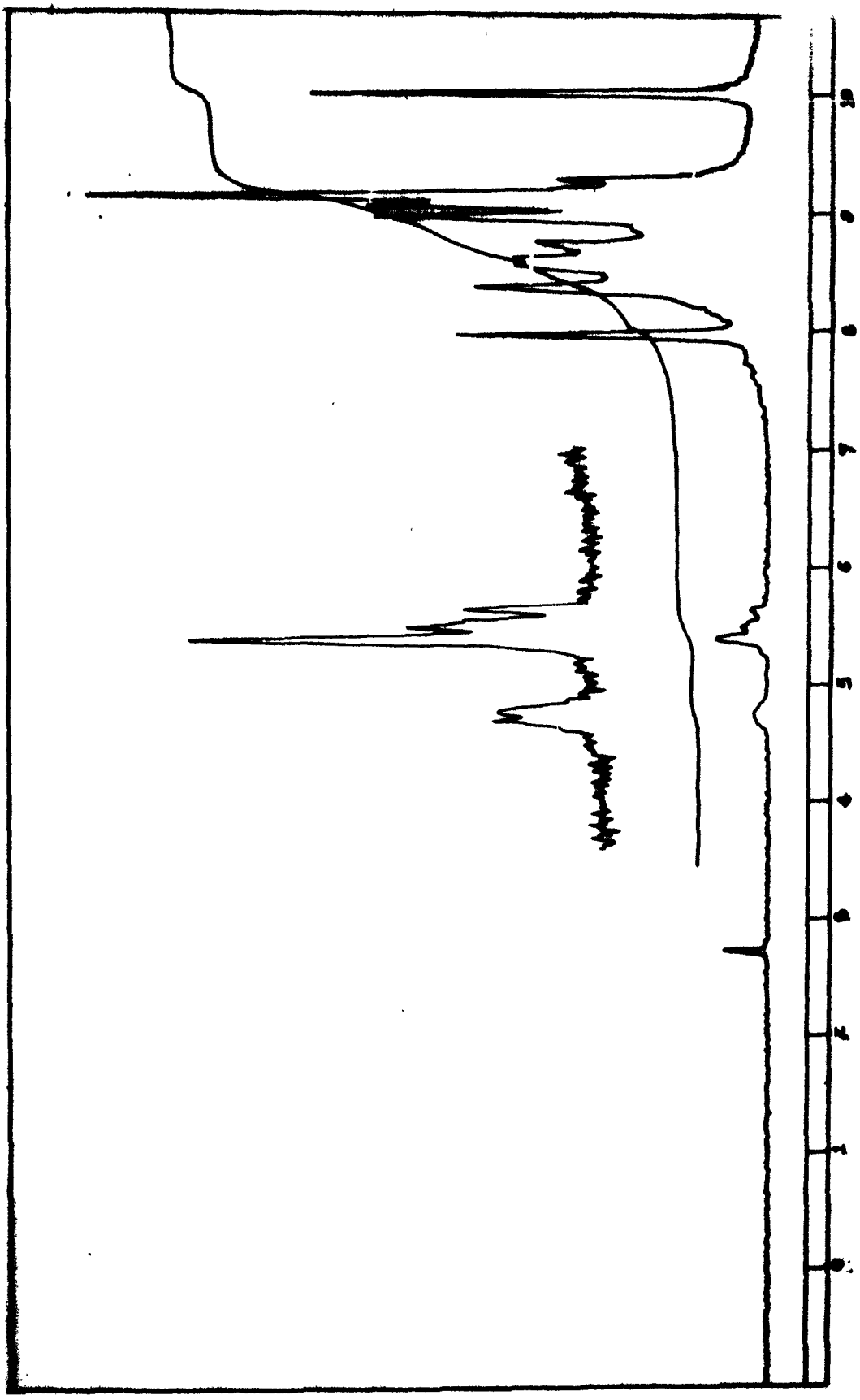


FIGURE- 20

The lowest field multiplet can only be assigned to an olefinic proton, presence of a double bond being further supported by broadish signal of the olefinic methyl at 8.20.

Before turning to the identification of the compound it is essential to dispose of the question of the presence or absence of an impurity. It was mentioned above that the spectrum of the compound resulting through attempted hydrogenation was helpful in deciding that the signal at 5.40 was that of an impurity. Owing to the known propensity of the triterpene carbon skeleton to backbone rearrangement during hydrogenation the methanolic solution of the compound was in one attempt exposed to hydrogen over palladium charcoal catalyst for only ten minutes. Work up produced a compound which, though it ran beside the starting material on TLC plates, stained much more weakly in iodine. This was interpreted as an indication of the disappearance of the double bond but the NMR spectrum of the compound turned out to be identical (Fig. 21) with that of the starting material in all respects except the absence of the broadish singlet which, as noted earlier, distorts the symmetrical signal of the proton under acetate oxygen. This triplet as evident from the NMR spectrum of the attempted hydrogenation product is exactly the duplicate of the one at 6.75 in the NMR spectrum of the alcohol. Since the NMR spectrum is otherwise unchanged the only conclusion to be drawn from this is that the impurity, was hydrogenated during the short exposure to hydrogen and thereafter

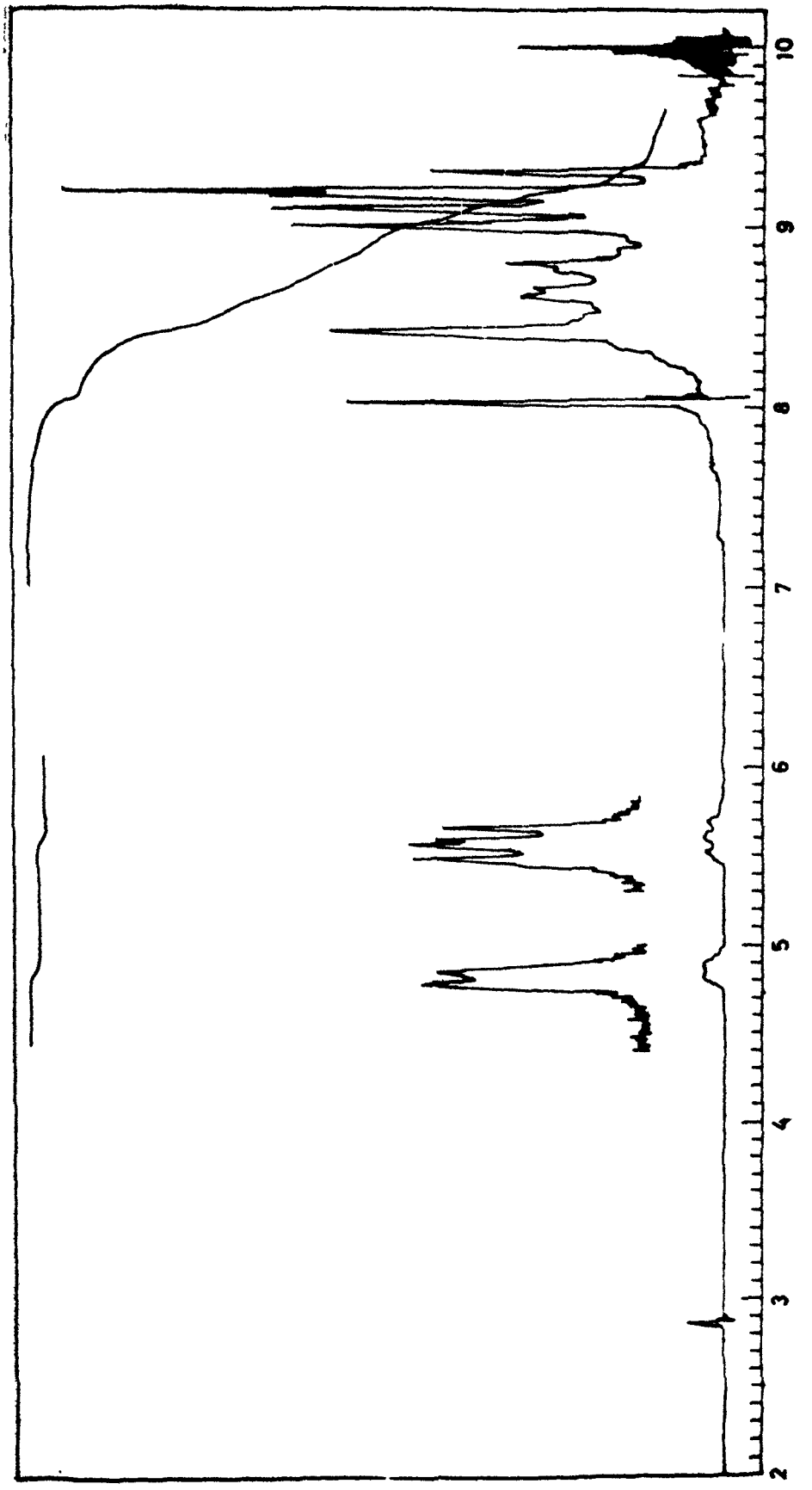
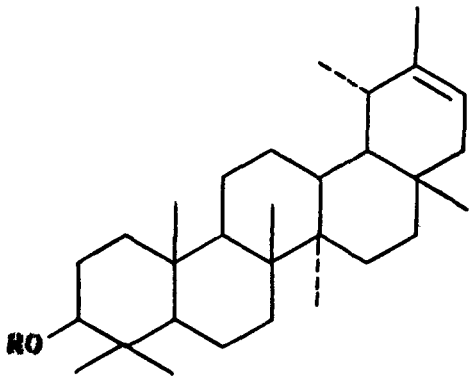


FIGURE- 21

becomes more soluble and does not crystallise with the main product. It is possible that the impurity was also responsible for the more pronounced staining of the triterpene before hydrogenation.

The data discussed so far, therefore, identifies the functional groups of the triterpene as one trisubstituted double bond, one secondary hydroxyl and an olefinic methyl. Since these functional groups also characterise pseudo-taraxasterol the triterpene inspite of the difference in melting point must be pseudo-taraxasterol (LXXII) as reported by Tewari et al.



(LXXII) a, R=H, Pseudo-taraxasterol
b, R=Ac, Pseudo-taraxasteryl acetate

The mass spectrum of the compound shows $M^{+\cdot}$ at 426 for the alcohol and 468 for the acetate. Loss of H_2O and acetic acid respectively from these gives the peak at m/e 408 which is prominent in the spectra of both compounds. Structurally the most useful fragmentation especially of pentacyclic triterpenes

is the retro-Diels-Alder fission of the molecule. Search for the fragment of this type focuses attention on fragments about half the molecular weight and in this region of the mass spectrum one is struck by the presence of ions at m/e 218 (relative intensity 231.8) and m/e 189 (1000). The ions are accompanied by other prominent ions at m/e 249 (156.0), 175 (203.0), 163 (103), 161 (177), 149 (199), 147 (149), 136 (221), 135 (299), 134 (143), 133 (175), 123 (1204), 121 (428), 119 (273), 109 (471), 107 (394), 95 (353), 93 (327), and 91 (316). The fragmentation pattern matches in every detail with that of pseudo-taraxasterol.[112]

Since pseudo-taraxasterol - specially its acetate-was isolated in fairly large amounts, it appeared worthwhile to study the course of some reactions of structural value with it. The reactions chosen were osmylation, SeO_2 oxidation and reaction with DMSO-Ac_2O. Osmylation occurred readily and gave a diol in good yield. The NMR spectrum of the product (Fig. 22) is devoid of the singlet of the methyl group at 8.20 and the multiplet of the proton at 4.75 instead there is a broad singlet at 6.75 integrating for two protons. The well defined triplet of the proton under the acetate oxygen at 5.40 indicates that the impurity responsible for its distortion is eliminated during work up and purification of the product. The mass spectrum of the diol shows $M^{\bullet+}$ at the expected value m/e 502 and hence the diol must be formulated as (LXXIII).

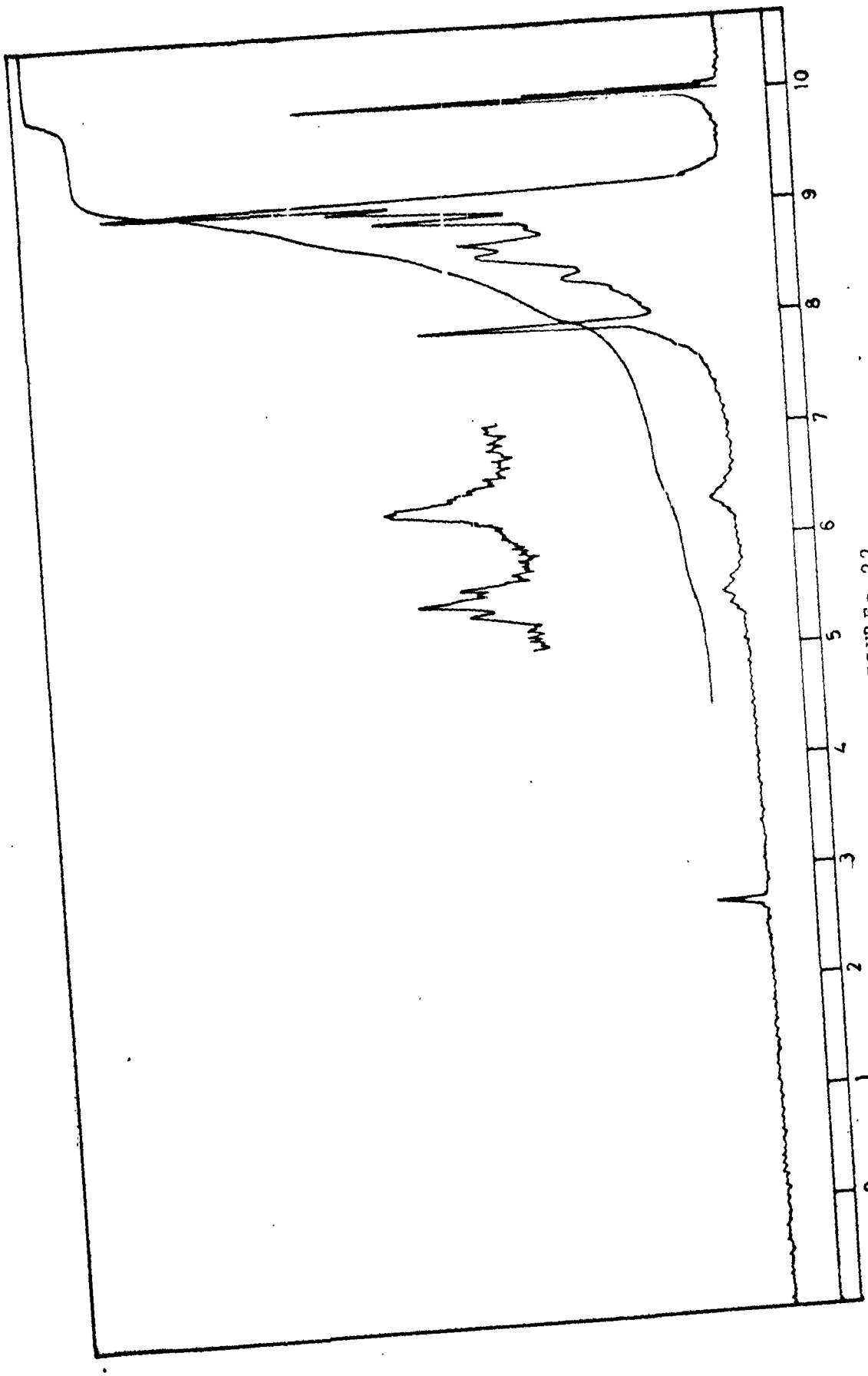
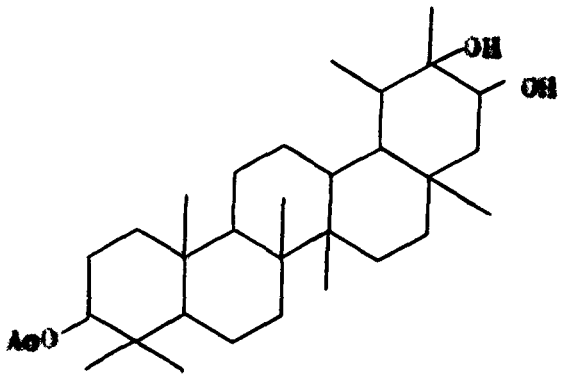
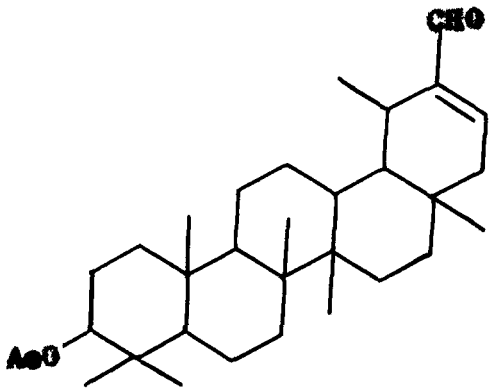


FIGURE- 22



(LXXIII)

SeO_2 oxidation was conducted in dioxane and gave the aldehyde (LXXIV). The identification is based on the disappearance of the singlet of the olefinic methyl and shift of 1 in the position of the olefinic multiplet and appearance of a singlet of the aldehydic proton at 9.60 (Fig. 23).



(LXXIV)

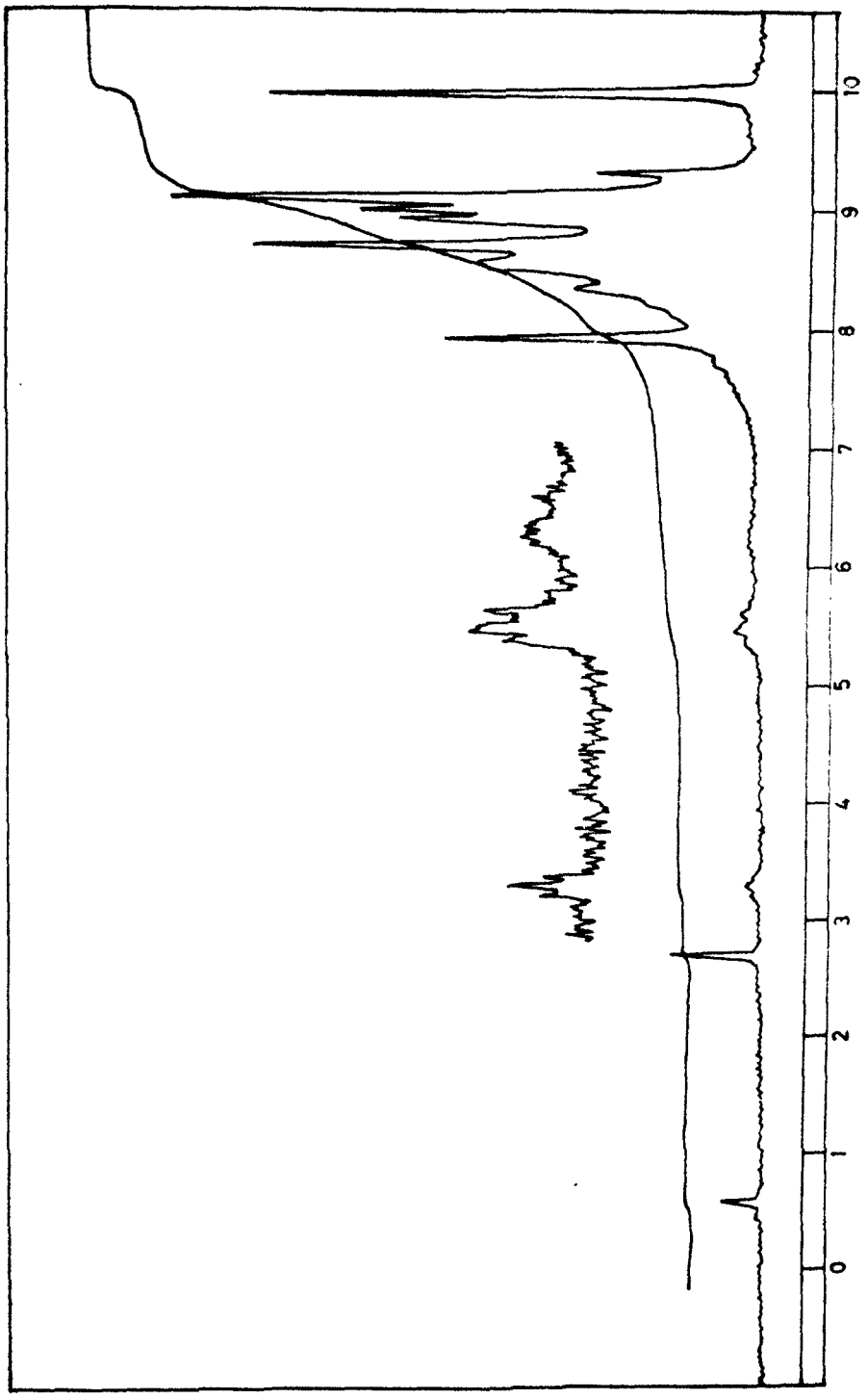
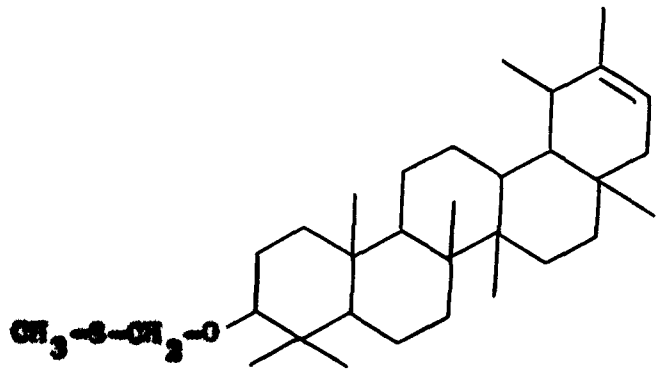


FIGURE- 23

The triterpene alcohol when subjected to Jones/Sarett oxidation inexplicably led to intricate mixture of products. However, the attempted oxidation of the molecule with acetic anhydride-dimethyl sulphoxide[113] gave neat results and the only product obtained was eventually assigned structure (LXXV). Comparison of its NMR (Fig. 24) with that of triterpene alcohol and acetate revealed that the pattern of the methyl and methylene resonances of this product resembles very much to the former. It shows the methyl singlet at 7.90, which could be attributed to a $S-CH_3$ group and a methylene singlet at 5.45 besides the multiplets of CH-OH and an olefinic proton at 6.92 and 4.95 respectively. Clearly the triterpene has undergone thiomethoxy methylation.



(LXXV)

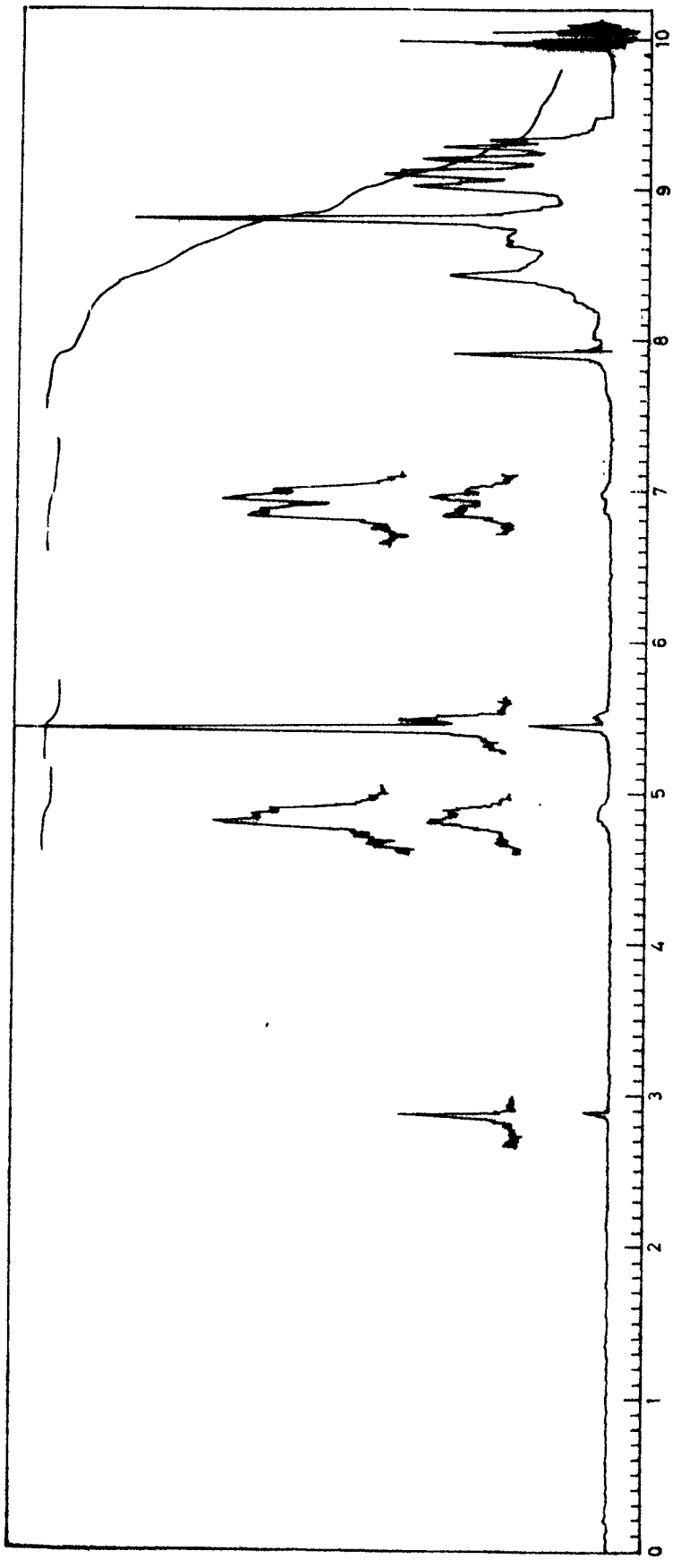


FIGURE- 24

Excoecharia agallocha Linn.

Excoecharia agallocha (N.O. Euphorbiaceae) was collected from Goa. 'The wealth of India'[114] ascribes several medicinal uses to it which are associated with the light yellow acrid latex with repulsive odour. It blisters the skin and is reported to cause blindness on contact with the eyes. It is used as a caustic in the treatment of ulcers. The juice boiled in oil is applied in rheumatism, leprosy and paralysis, and the oil obtained through destructive distillation is used in Burma in the treatment of cutaneous affections. It is used as a fish poison and as an adjunct to arrow poisons. The leaves are also poisonous and the tree is listed among those occasionally poisonous to stock. While most of these attributes may be fictitious, there seems little doubt from the above discussion that the plant contains some toxic principle.

The petroleum ether extract of the plant was found to contain two substances, one a chalcone, readily identified as 2',4',6',4-tetramethoxy chalcone (LXXVI) on the basis of NMR singlets at 6.26, 6.28 and 6.34 (4 x OMe), 3.28 (2 x ArH) and A_2B_2 and AB doublets at 2.66, 3.28 (ArH) and 2.96, 3.35 (=CH) respectively (Fig. 25) and comparison with a synthetic sample.

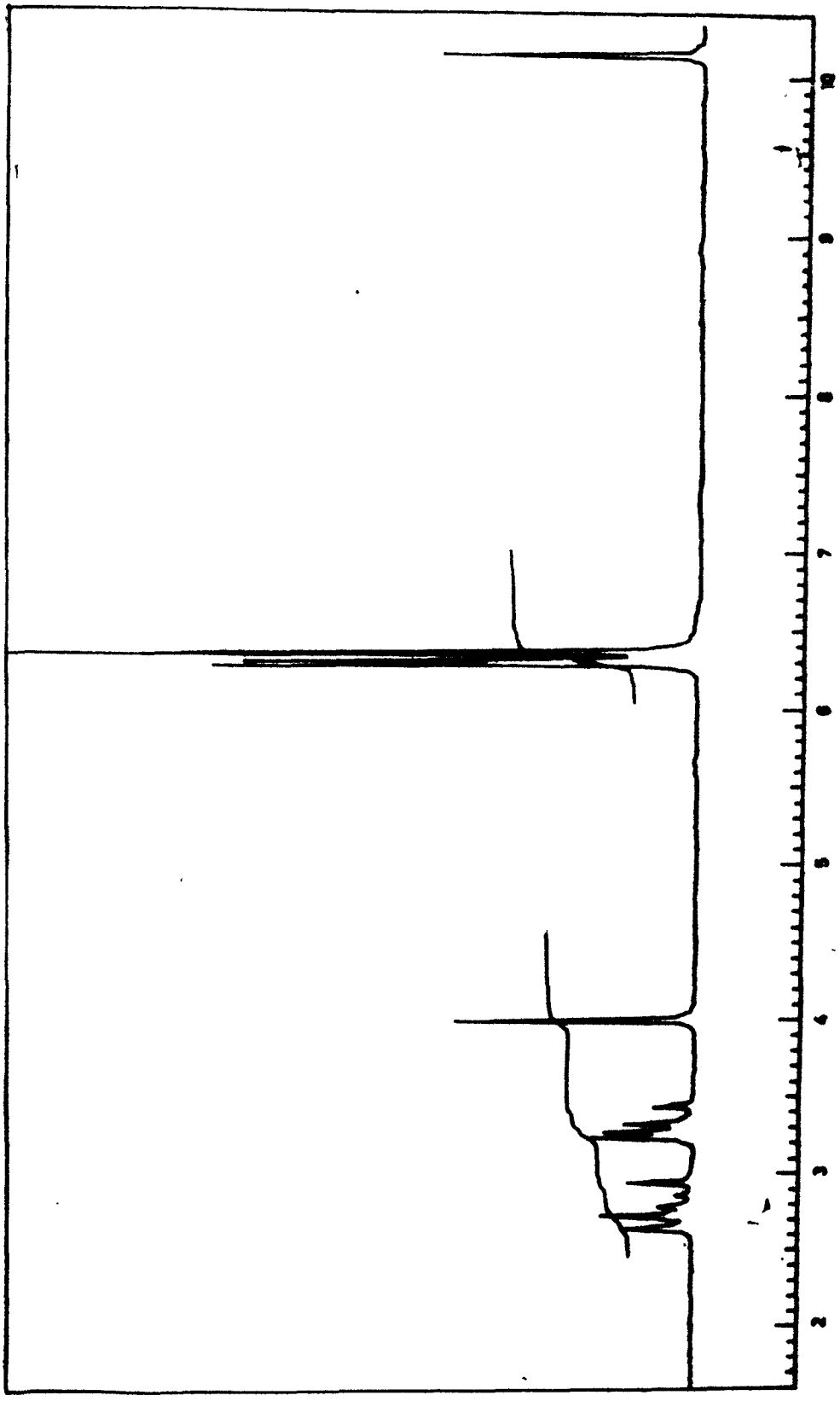
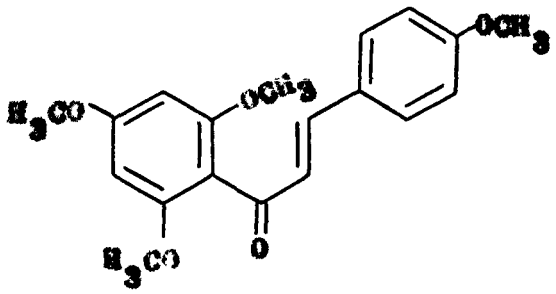
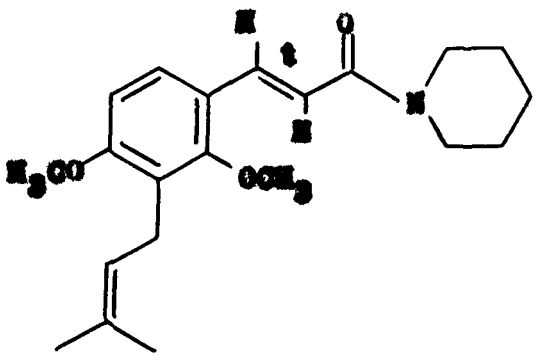


FIGURE- 25



(LXXVI)

The other compound was an oil the purity of which was established by TLC. The IR spectrum of the oil (Fig. 26) shows it to be aromatic in nature and the carbonyl band at 1650 cm^{-1} is indicative of conjugation. The mass spectrum of the oil shows M^+ at m/e 343 and hence presence of nitrogen is essential. In keeping with the assumption the compound gave positive Dragendorff test. The UV spectrum also offers indication of conjugation showing maxima at 350, 285 and 235 nm. The NMR spectrum (Fig. 27) which leads to structure (LXXIX) for the compound establishes the presence of a γ,γ -dimethylallyl side chain. The gem - dimethyls of this side chain are covered over



(Lxxix)

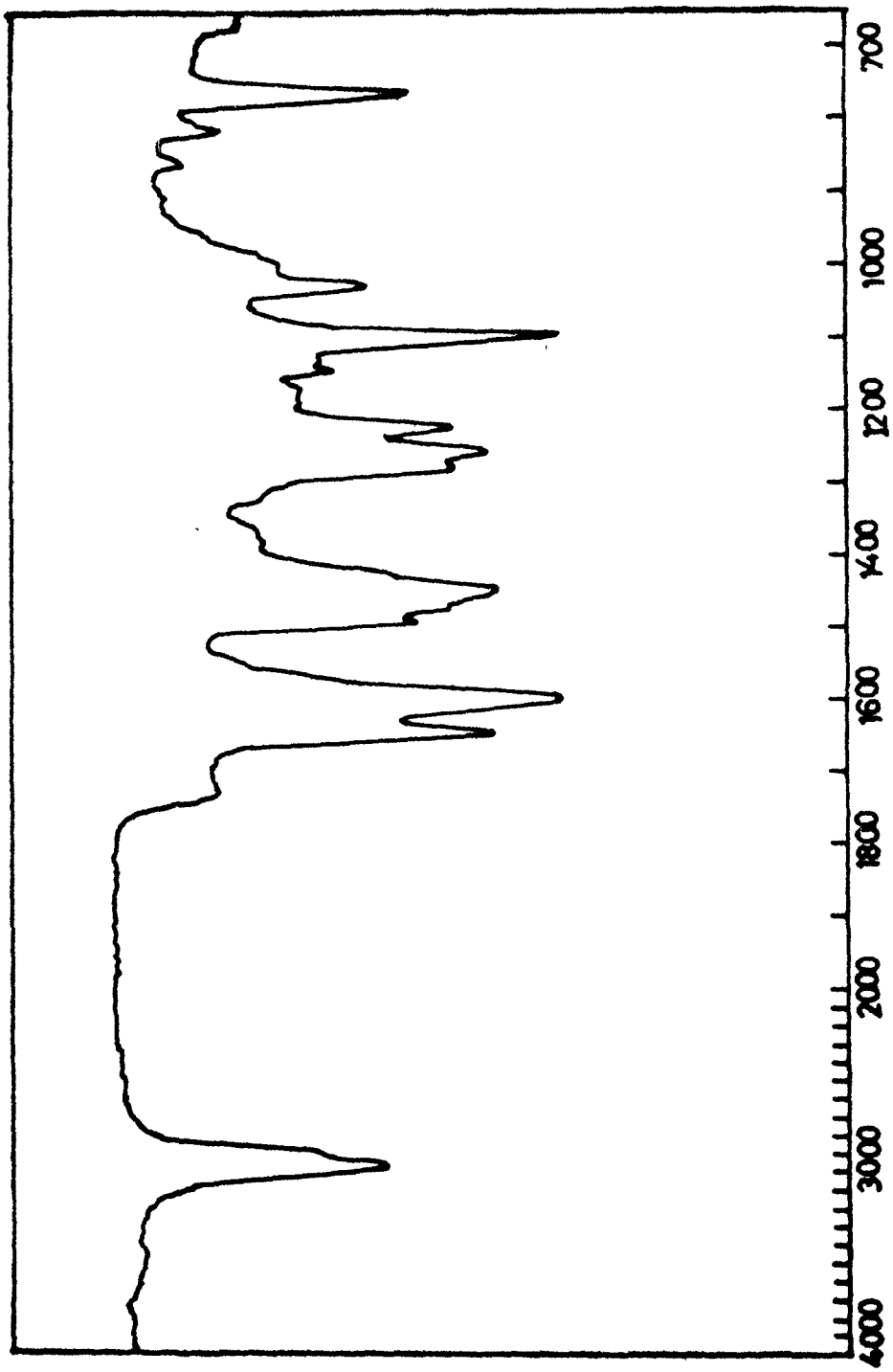


FIGURE- 26

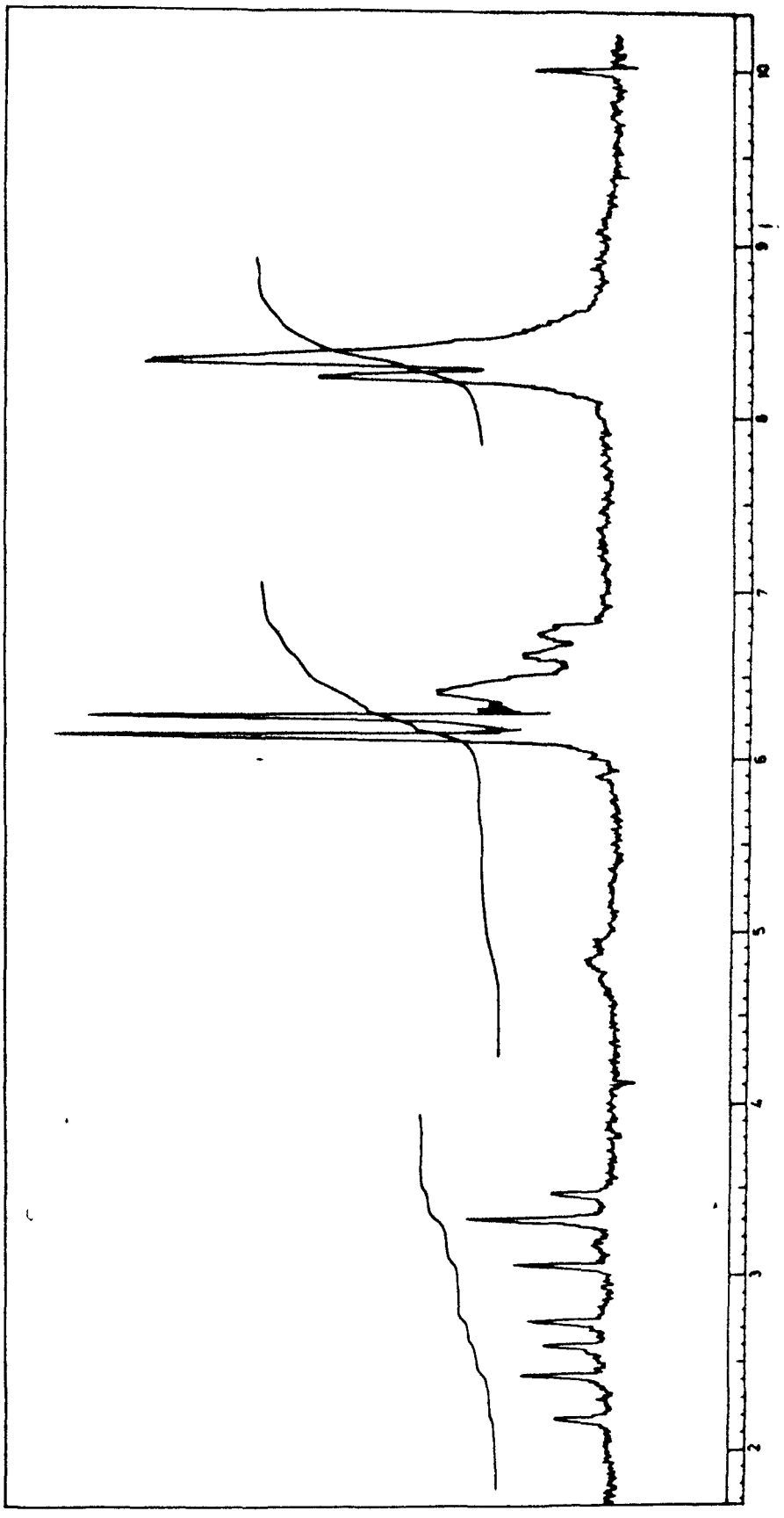


FIGURE- 27

by signals of other protons but resonances of the methylene and methine protons are clearly discernible. The compound has two methoxyl groups which give rise to signals at 6.28 and 6.38. Between the methoxyl singlets and the doublet of benzylic methylene of the side chain the NMR spectrum shows a broad signal which, taking the integration of methyls as reference, appears to contain resonances of four protons. The chemical shift of these protons is that required for methylenes bonded to nitrogen[115] and presence of the piperidine system in the molecule is thus suggested. The integration of protons resonating above τ is in accord with the assumed structural features and works out to twelve hydrogens from three methylenes of the piperidine ring and two gem-dimethyls of the prenyl side chain. Any doubt as to the presence of gem-dimethyl groups is removed by the spectrum obtained in $CDCl_3$ containing a few drops of deuterated benzene which shows methyls singlets distinctly at 8.32 and 8.48 (Fig. 28).

The most deshielded proton in the spectrum in $CDCl_3$ gives rise to a doublet at 3.38 (J=16 Hz), the counterpart of this doublet appears at 3.30. The large coupling constant and the positions of these doublets are understandable only if the concerned protons are olefinic, trans to each other and the double bond is conjugated with the carbonyl. Assembly of the structural features discussed above leads to the part structure (LXXVII).

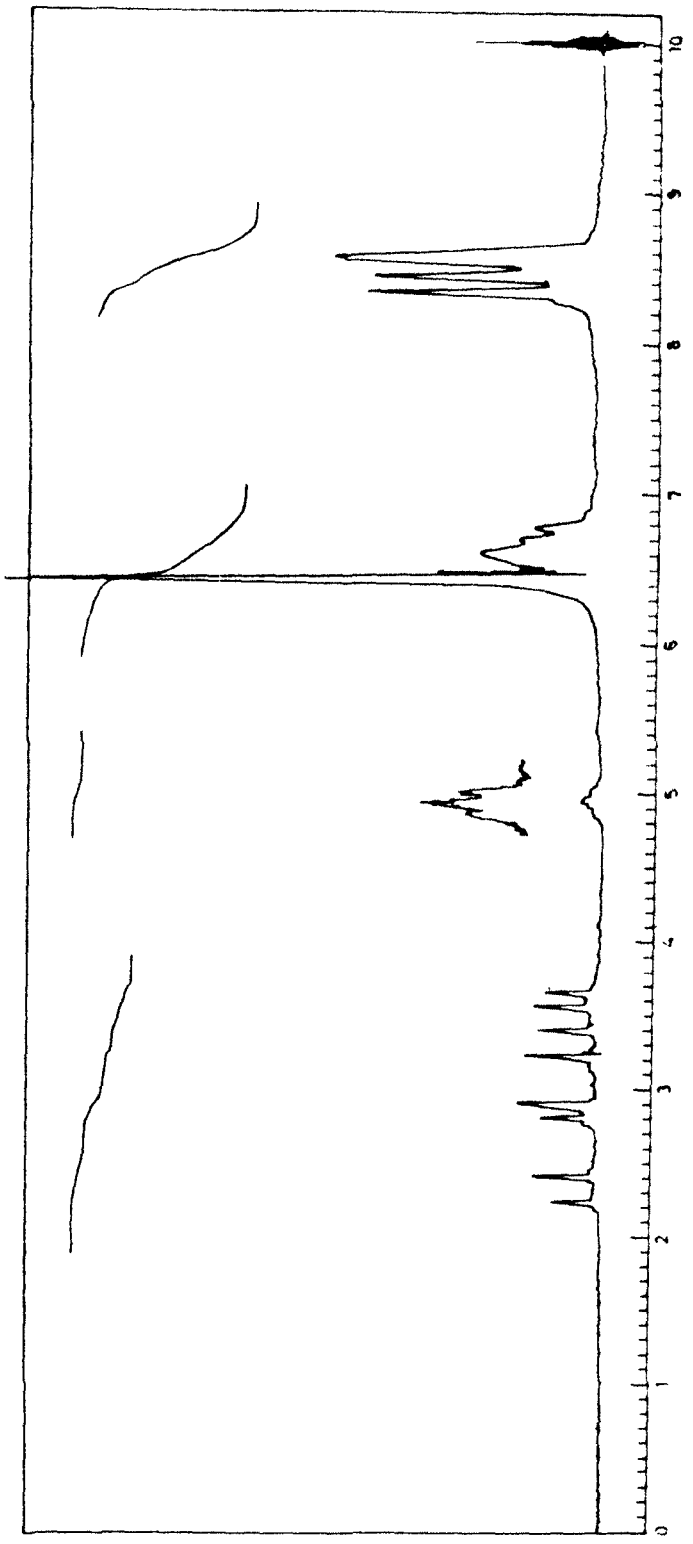
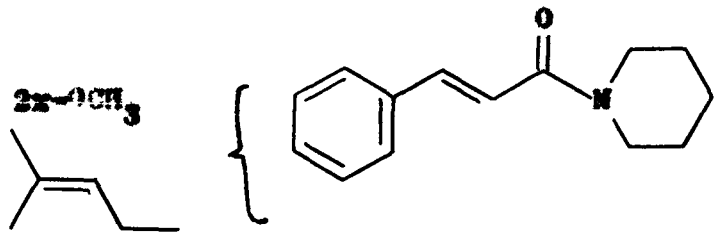
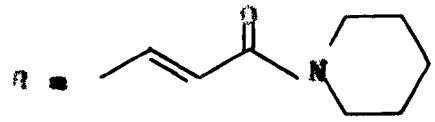


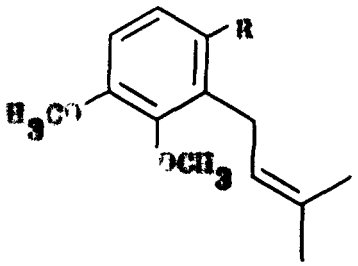
FIGURE- 23



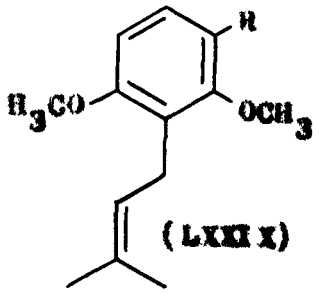
(LXXVII)

With regard to the substitution of the benzene ring only those arrangements need be considered which allow two adjacent protons since the ortho coupled doublets of these are unmistakable at 3.30 and 3.45 (J=9 Hz). With this limitation structures (LXXVIII to LXXXIII) are possible.

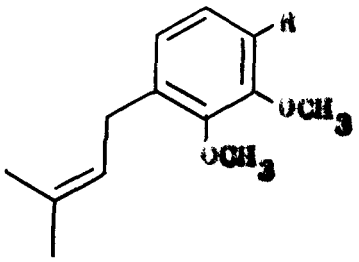




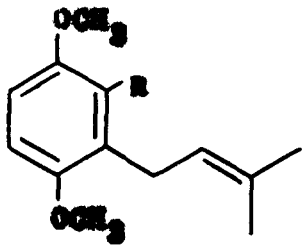
(LXXVIII)



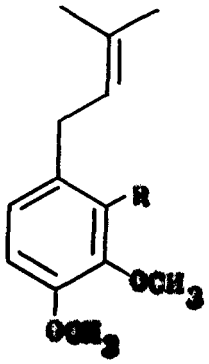
(LXXIX)



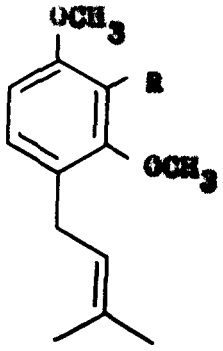
(LXXX)



(LXXXI)



(LXXXII)

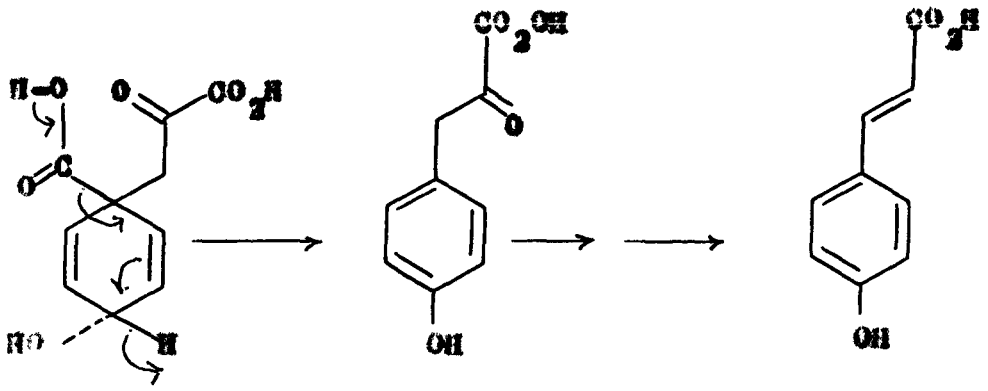


(LXXXIII)

In reaching a decision regarding the correct structure the position of the doublets of two aromatic protons is helpful. The doublet at 2.90 appears at a value only slightly different from that of protons in unsubstituted benzene, 2.70. But the doublet at 3.45 must be attributed to a proton which is either flanked by two methoxyl groups or bears ortho and para relationship to these. In the spectrum with benzene added the proton at 3.45 is found to have suffered a shift of 0.17, thus further indicating that it is adjacent to methoxyl group (Fig. 28).

The effect of substituents on benzenoid protons was studied by Ballantine and Pillinger[8] and the table 2 summarising their conclusions is given in theoretical section. According to it a proton in ortho and para relationship to methoxyls should resonate at 3.55. The chemical shift of the most shielded aromatic proton is almost the same as that required and hence the choice has to be made out of structures (LXXIX) and (LXXXIII) which has such a proton.

While spectroscopic evidence does not allow a distinction to be made between these two, (LXXIX) is to be preferred on biogenetic grounds for derivatives of para-hydroxy cinnamic acid (p-coumaric acid) are by far the most abundant in nature. This has its basis in the biogenesis from prephenic acid (LXXX) as outlined in detail earlier and for the particular step reproduced again below.



(LXXX)

The structure is thus completely settled in formula (LXXV). Since the compound contains an amidic nitrogen its insolubility in acids is understandable, its alkaloidal nature being shown only by positive Dragendorff's test.

EXPERIMENTAL

Calophyllum wightianum T. Anders

Isolation: Air dried heartwood of the plant (5 Kg) was chopped into small pieces and exhaustively extracted with hot benzene. The extracts were reduced to a small volume and combined. Removal of the solvent under reduced pressure gave a viscous mass (50 g) which was adsorbed on silica gel (100 g) and chromatographed. Elution with petroleum ether afforded only fatty material which was not further studied. Further elution with petroleum ether-benzene in the proportions 1:1, 1:2 and benzene yielded three compounds A, B and C respectively.

COMPOUND A

It was crystallised from methanol (1.2 g), m.p. 135-36°C, identified as β-sitosterol by comparison with an authentic sample (mixed m.p., I.R., TLC).

β-Sitosterol acetate

Was prepared by heating β-sitosterol (0.2 g) with acetic anhydride and pyridine (2 ml each) on a water bath for two hours, and worked up to give needles, m.p. 127-28°C (0.18 g).

β-Sitosterol benzoate

Was obtained by refluxing β-sitosterol (0.2 g) with pyridine and benzoyl chloride (1.2 ml) for 1 hr. After addition of 20 ml of 5% sodium bicarbonate solution and cooling, the solid was filtered and chromatographed over silica gel (20 g). Elution with benzene afforded the benzoate, m.p. 146°C (0.1 g).

COMPOUND B

The petroleum ether-benzene (1:2) eluates were combined and processed. The solid obtained (0.7 g) was crystallised from chloroform-methanol, m.p. 197-200°C. It was identified as β-amyrin through comparison with an authentic sample (mixed m.p., Co-TLC).

β-Amyrin acetate

β-Amyrin (100 mg) was heated on a water bath for four hours with acetic anhydride and pyridine (1 ml each and the acetate worked up and crystallised from methanol, m.p. 240-41°C (90 mg).

COMPOUND G (XLIV)

The solid mass obtained from the column was not homogeneous and hence rechromatographed over silica gel and repeatedly crystallised with petroleum ether-benzene to give yellow needles (500 mg), m.p. 163°C.

Spectral data

$M^{+\cdot}$ m/e 450.2493 analysed for $C_{28}H_{34}O_5$, νmax (Nujol) 3320, 1700, 1643, 1590, 1190 and 1080 cm^{-1}; λ max (MeOH) 230, 259, 300 and 330 nm; NMR (220 MHz, $CDCl_3$) -3.23 (1H, s-OH), 3.66 (1H, s, ArH), 3.72 (1H, m, -OH), 4.65 (1H, t, $-CH_2-CH=C\langle$), 5.15 (2H, t, $2x-CH_2-CH=C\langle$), 6.50 (2H, d, $Ph-CH_2-CH=C\langle$), 6.94 (2H, q, J=13.5, 8.5 Hz, $2x-CH_AH_B-CH=$), 7.08 (2H, t, $-\overset{O}{\overset{\|}{C}}-CH_2-CH_2-$), 7.30 (2H, q, J=13.5, 7.7 Hz, $-CH_AH_B-CH=$), 7.36(2H, t, $-\overset{O}{\overset{\|}{C}}-CH_2-CH_2$), 8.10 and 8.18 (3H each, s, $Ph-CH_2-CH=C(CH_3)_2$), 8.40 and 8.48 (6H each, s $C-\left[CH_2-CH=(CH_3)_2\right]_2$), 8.70 (6H, s, $-(CH_2-)_n$). M.S. M^+ m/e 450.2493 (40%), 396 (65), 394 (70), 381 (100).

Acetylation of xanthiosone

XLIV (50 mg) was mixed with acetic anhydride/pyridine (0.5 ml each) and kept overnight at room temperature. The acetate was worked up by addition of cold water and extraction

with ether. The ethereal solution was washed with dil. HCl, aq. $NaHCO_3$ and water, dried on anhydrous Na_2SO_4 and ether evaporated. The product obtained was chromatographed over silica gel. Elution with benzene afforded an oil (30 mg) which was found to be homogenous on a TLC plate.

Spectral data

$M^{+\cdot}$ m/e 492 analysed for $C_{30}H_{36}O_6$; ν_{max} (Nujol) 3410, 1770, 1645, 1620, 1190, 1175 cm^{-1}; NMR (60 MHz, $CDCl_3$) -3.23 (1H, s, -OH), 3.35 (1H, s, Ar-H), 4.90 (1H, m, $-CH_2-CH=C\langle$), 5.20 (2H, m, 2x $-CH_2-CH=C\langle$), 6.70 (2H, d, Ph-CH_2-$CH=C\langle$), 6.90-7.50 (9H, m, 4x-CH_2-$CH=C\langle$), 7.70 (3H, s, $-OC-CH_3$), 8.20 and 9.30 (3H each, s, $Ph-CH_2-CH=C(CH_3)_2$), 8.40 and 8.50 (6H each, s, $2x-CH_2-CH=C(CH_3)_2$), 8.70 (6H, s, $-(CH_2)_n$).

Methylation of wightianone

XLIV (50 mg) was dissolved in methanol (10 ml), excess of ethereal diazomethane added to it and the solution left for 36 hours to refrigerator. The solvent was evaporated and the residue chromatographed over silica gel. Elution with benzene afforded pale yellow needles (45 mg), m.p. 98-99°C.

Spectral data

$M^{+\cdot}$ m/e 464, analysed for $C_{29}H_{36}O_5$; ν max (Nujol) 3400, 1720, 1660, 1650, 1610, 1585, 1190, 1175 cm^{-1}; NMR (220 MHz, $CDCl_3$), 3.25 (1H, s, -OH), 3.70 (1H, s, ArH), 4.65 (1H, t, $-CH_2-CH=C\langle$), 5.20 (2H, t, 2x $-CH_2-CH=C\langle$), 6.10 (3H, s, $-OCH_3$), 6.30 (2H, d, $Ph-CH_2-CH=C\langle$), 6.84 (2H, q, J=13.5, 8.5 Hz, 2x $-CH_ACH_B-CH=C\langle$), 7.12 (2H, t, $-\overset{O}{\overset{\|}{C}}-CH_2-CH_2-$), 7.30 (2H, q, J=13.5, 7.7 Hz, $-CH_ACH_B-CH=C\langle$), 7.40 (2H, t, $-\overset{O}{\overset{\|}{C}}-CH_2-CH_2-$), 9.10 and 9.18 (3H each, s, $Ph-CH_2-CH=(CH_3)_2$, 2.40 and 2.48 (6H each, s, $C-\left[CH_2-CH=C(CH_3)_2\right]_2$), 8.70 (6H, s, $-(CH_2-)_n$).

Hydrogenation of wightianone

XLIV (80 mg) was dissolved in methanol (15 ml) and palladium charcoal (20 mg) added to it. Hydrogenation was complete in six hours. The catalyst was filtered off and solvent evaporated. The hydrogenation product was passed through a column of silica gel and crystallised from acetone-petroleum ether to afford cream coloured needles (30 g), m.p. 158°C.

Spectral data

$M^{+\cdot}$ m/e 456, analysed for $C_{28}H_{40}O_5$; ν max (Nujol) 1700, 1645, 1610, 1590 cm^{-1}, λ max (MeOH) 235, 260, 300 nm. NMR (60 MHz, $CDCl_3$)-3.26 (1H, s, -OH), 3.70 (2H, brs, ArH and -OH),

7.05 (2H, m, Ph-$\underline{CH}_2$-CH_2-), 7.10-7.90 (14H, m, 7x-CH_2-), 8.95 and 9.10, (3H each, s, 2x-CH_3), 9.12 and 9.18 (6H each, s, 4x-CH_3), 9.25 (6H, s, -$(CH_2)_n$-).

Urea treatment of wightianone

Compound (0.1 g) was crystallised several times from methanol saturated with urea at 40°C. After all the urea had been crystallised out the methanolic solution was evaporated and the residue crystallised from benzene-petroleum ether to give needles (0.05 g), m.p. 130°C. The NMR spectrum of the product (220 MHz, $CDCl_3$) was almost identical with the original compound but for the intrusive singlet at 8.70, the intensity of which was reduced considerably. NMR (220 MHz, C_6D_6): -4.05 (1H, s, -O$\underline{H}$), 3.78 (1H, s, Ar$\underline{H}$), 3.82 (1H, m, -O$\underline{H}$), 4.58 (1H, t, -CH_2-$\underline{CH}$=C$\langle$), 4.92 (2H, t, 2x-CH_2-$\underline{CH}$=C$\langle$), 6.46 (2H, d, Ph-$\underline{CH}_2$-CH=C$\langle$), 6.50 (2H, q, J=13.5, 8.5 Hz, 2x -$CH_A\underline{CH}_B$-CH=C$\langle$), 6.92 (2H, q, J=13.5, 7.7 Hz, -$\underline{CH}_A CH_B$-CH=C$\langle$), 7.67 (4H, t, -C(=O)-$\underline{CH}_2$-$\underline{CH}_2$-), 8.28 (3H, s, =C(-CH_3)-), 8.40 (9H, s, 3x =C(-CH_3)-), 8.48 (6H, s, 2x =C(-CH_3)-).

Regeneration of the clathrate

Wightianone (300 mg) obtained on purification with urea was dissolved in methanol containing authentic palmitic acid (200 mg) and left for crystallisation to give yellow needles

melting at 160°C. Its NMR spectrum differed from that of the natural clathrate only in the intensity of the intrusive singlet at 9.70 which was somewhat higher.

Methylation of the fatty acids

The urea crystallisate from the methanolic solution of wightianone was dissolved in H_2O extracted several times with ether. The extracts were combined and solvent reduced to a volume of about 10 ml, methylated with an excess of ethereal diazomethane. GLC analysis of the methyl ester showed it to be methyl palmitate with a little of methyl myristicate and methyl stearate.

Synthesis of the 1,2,3,4-tetrahydroxanthone (XLVI)

(a) 1-Morphiline-1-cyclohexene

A solution of cyclohexanone (14.7 g, 0.15M), morpholine (13.7 g, 0.16M) and p-toluene sulphonic acid (1.5 g) in toluene (30 ml) was heated to boiling in a 500 ml round bottomed flask to which was attached a Dean-Stark Trap. The separation of water which began at once continued for 4-5 hours. After this period toluene was distilled off from the mixture at atmospheric pressure and 1-morphiline-1-cyclohexene obtained as a colourless liquid, b.p. 118-120°C/10 mm (20 g).

(b) Condensation of enamine with salicyladehyde

To a solution of salicyladehyde (3.8 g) in anhydrous benzene (2 ml) was added in one portion an equimolar quantity of enamine (3.3 g) in benzene (2 ml). The resulting solution was allowed to stand in a loosely stoppered flask for a period of 24 hrs. The solvent was removed in vacuum to afford a yellow viscous oil.

(c) Sarett oxidation

To a stirred suspension of CrO_3 pyridine complex (20 g in 100 ml) in pyridine cooled in an ice bath was added dropwise a solution of the above pale yellow oil (10 g) in pyridine. On completion of addition the mixture was stirred with cooling for another 2 hrs and was allowed to stand overnight at room temperature. The mixture was poured into ice water and the dark brown slurry was extracted with ether. The combined ether extracts were washed thrice with water, dried over anhydrous Na_2SO_4, filtered and evaporated. The compound was purified by passing through a column of silica gel and the resulting solid product crystallised from petroleum ether to yield white prisms, m.p. 102-103°C. NMR (60 MHz, $CDCl_3$), 1.90 (1H, q, J=9, 2 Hz, C-1H), 2.65 (1H, q, J=9, 2 Hz, C-8H), 2.75 (1H, q, J=9, 2 Hz, C-3H), 2.95 (1H, q, J=9, 2 Hz, C-4H), 7.50 (4H, m, C-6 and C-7-CH_2-), 8.28 (9H, m, C-5 and C-8-CH_2-).

Tinospora malabarica Miers

Isolation

Air dried stem wood (3 Kg) of the plant was cut into small pieces and extracted with petroleum ether in a Soxhlet. Evaporation of the solvent left a gummy mass (50 g) which was dissolved in petroleum ether-benzene (1:1, 200 ml) and left in a refrigerator for a week when a light green solid was deposited at the bottom of the flask. The solid mass was filtered, adsorbed on silica gel and subjected to column chromatography. Elution with petroleum ether gave only oily products which were discarded. Further elution with petroleum ether-benzene (1:1) and pure benzene gave two compounds A and B respectively.

The defatted heart wood was then exhaustively extracted with ethanol and the solvent removed under reduced pressure. The black sticky mass (300 g) was extracted with ethyl acetate (3 x 800 ml) and the dried ethyl acetate extract chromatographed on silica gel. Elution with chloroform-petroleum ether (2:1) and then with $CHCl_3$ supplied solid products C and D respectively.

TLC of the ethyl acetate insoluble alcohol extract revealed the presence of only one component which was purified by repeated crystallisation with methanol-water. The compound,

m.p. 228–30°C, was identified as gillein through comparison with an authentic sample.

COMPOUND A

It was crystallised from petroleum ether (1 g), m.p. 79–80°C, and identified as heptacosanol by comparison with an authentic sample.

Heptacosanol acetate

It was prepared by treating heptacosanol (100 mg) with acetic anhydride-pyridine (1 ml each). Usual work up of the reaction mixture and crystallisation from chloroform-methanol gave the acetate (90 mg), m.p. 66–67°C.

COMPOUND B

Benzene eluates obtained on chromatography of the extract were processed to yield a solid, crystallised from methanol, (800 mg), m.p. 135–36°C. It was identified as β-sitosterol through comparison with a sample isolated earlier from Calophyllum wightianum.

COMPOUND C

The solid mass eluted from the column with petroleum ether-chloroform (2:1) was purified further by repeated column chromatography over silica gel and crystallisation from benzene to yield the pure compound (200 mg), m.p. 162°C.

Spectral data

$M^{+\cdot}$ -OMe m/e 311, analysed for $C_{18}H_{15}O_5$; ν max (Nujol) 1670, 1660, 1505, 915, 800 cm^{-1}; λ max ($CHCl_3$) 250, 275 nm; NMR (100 MHz, $CDCl_3$), 2.05 (1H, d, J=9 Hz, ArH-6'), 2.42 (1H, dd, J=9 Hz, ArH-2), 2.45 (1H, d, J=2 Hz, ArH-6), 3.13 (1H, d, J=9 Hz, ArH-5), 3.48 (1H, dd, J=9,2 Hz, ArH-4'), 3.70 (1H, d, J=2 Hz, ArH-2'), 3.95 (2H, s, $-O-CH_2-O-$), 4.90 (1H, q, J=7 Hz, $>CH-CH_3$), 6.20 and 6.55 (2H each, s, $2x-OCH_3$), 8.38 (3H, d, J=7 Hz, $>CH-CH_3$); M.S. 311 (100), 163 (98), 152 (10), 149 (40), 138 (48), 136 (7), 122 (15).

Synthesis of tinosporinone (LVII)

(A) Milletenone (LX)

(1) 2,4-Dimethoxy acetophenone

This was prepared by methylating resacetophenone using $(CH_3)_2SO_4$ and NaOH in the same proportions as used by Spath and Koral[116] in the preparation of 2,4-dimethoxy benzoic acid.

Resacetophenone (7.7 g) was dissolved in aqueous alkali (10 ml) prepared by dissolving NaOH (32.6 g) in water (65 ml). Dimethyl sulphate (64.4 ml) and the remaining portion of the sodium hydroxide solution were added alternately to the vigorously stirred solution of resacetophenone. The hot reaction mixture was allowed to become acidic after each addition of dimethyl sulphate. The solution was finally made strongly alkaline by the addition of the last portion of the NaOH solution and heated for one hour on a boiling water bath. It was then cooled, diluted with water and extracted thrice with ether (50 ml portion each). The ether was dried (Na_2SO_4) and evaporated to give 6.5 g (72%) of the dimethyl ether.

(ii) <u>Piperonaldehyde</u>

DMSO (90 ml) and methylene iodide (4 ml) was added to a solution of 3,4-dihydroxybenzaldehyde (7 g) in which was added dropwise aqueous NaOH (5 g in 10 ml H_2O). The vigorously stirred mixture was heated on a water bath for 6 hrs., cooled, diluted with water (500 ml) and extracted exhaustively with ether till the ether extract was colourless. The dark brown muck which separated during this extraction and interfered with separation of the ether layer was removed by centrifugation. The combined ether extract (1 liter) was dried (Na_2SO_4) and ether evaporated to yield piperonaldehyde (3.5 g), yield 46%.

(iii) Piperonylic Acid

This was prepared from piperonaldehyde according to the procedure given in Organic Synthesis (Vol. II, p. 38).

An emulsion of piperonal (6 g, 0.04M) in water (150 ml) placed in a 500 ml round bottomed flask was heated to 70-80°C on a water bath and an aqueous solution of potassium permanganate (9 g in 180 ml) was added to it over a period of 45 minutes with vigorous stirring. The stirring and heating were continued for an hour longer, at the end of which time the permanganate was reduced. Enough aqueous potassium hydroxide solution (10%) was added to make the reaction mixture alkaline. The mixture was filtered while hot and the manganese dioxide washed with hot water (3 x 25 ml). The filtrate was acidified with conc. HCl and the precipitated piperonylic acid filtered, washed with cold water and dried. It was crystallised from ethanol to yield colourless needles (6 g), m.p. 224-25°C.

(iv) Methyl piperonylate

The above acid (3.5 g) in methanol (50 ml) was methylated with ethereal diazomethane. The oily product (3.2 g) crystallised in the refrigerator and was used as such.

(v) Condensation of 2,4-dimethoxy acetophenone with methyl piperonylate

Sodium (1 g) was melted under dry toluene and made into fine granules by vigorously shaking the flask. The toluene was decanted off and the sodium washed thrice with (25 ml) portions of dry ether and finally covered with ether (50 ml). Absolute alcohol (2 ml) was added and the solution refluxed for 4 hrs. A solution of 2,4-dimethoxy acetophenone (3 g) and methyl piperonylate (3 g) in dry ether (50 ml) added to the suspension of sodium ethoxide in ether and refluxing continued for further 4 hrs. The reaction mixture was worked up by addition of water, separation of the ether layer followed by extraction of the aqueous phase with ether. The residue obtained on evaporation of the combined ether extract was chromatographed over silica gel (50 g). The light petroleum ether-benzene (1:4) eluate gave the dibenzoylmethane, millettenone (LXI), yield 0.35 g, m.p. 138° (identical with an authentic sample, TLC, NMR).

(B) C-methylation of millettenone (LXI)

A mixture of millettenone (0.1 g), anhydrous K_2CO_3 (0.2 g), methyl iodide (1 ml) and dry acetone (10 ml) was refluxed on a water bath for four hours. The progress of the reaction was checked by TLC. On completion of the reaction the solution was filtered and the solvent removed under reduced pressure. The

solid obtained was crystallised from methanol, m.p. 182°C (0.09 g) which was identical with the natural product mixed m.p., I.R., TLC and NMR).

COMPOUND D

The combined $CHCl_3$ eluates was evaporated and the solid crystallised from methanol as colourless needles (300 mg), m.p. 182°C.

Spectral data

$M^{+\cdot}$ m/e 368, analysed for $C_{21}H_{20}O_6$; νmax (KBr) 1640, 1600, 1610; λmax (MeOH) 270, 320 nm; NMR (100 MHz, $CDCl_3$), 3.20 (2H, dd, J=9, 2 Hz, Ar<u>H</u>-2',6'), 3.05 (2H, dd, J=9, 2 Hz, Ar<u>H</u>-3',5'), 3.25 (1H, s, Ar<u>H</u>-8), 3.45 (1H, s, <u>H</u>-3), 3.90 (1H, m, $-CH_2-\underline{CH}=CH_2$), 4.90 (2H, m, $-CH_2-CH=\underline{CH}_2$), 5.35 (2H, d, $-\underline{CH}_2-CH=CH_2$), 6.05, 6.12 and 6.16 (3H each, s, 3x$-OCH_3$); M.S. 368 (62), 353 (100), 337 (65), 328 (60), 325 (70), 323 (100), 313 (90), 312 (75), 311 (95), 281 (80), 195 (40), 167 (100), 153 (80), 132 (80).

Hydrolysis of (LXVII)

LXVII (100 mg) was refluxed with methanolic HCl (10 ml 5%) for an hour on a water bath. The reaction mixture was

diluted with cold water and extracted with ether. Evaporation of the solvent gave a solid which was crystallised from chloroform to give (LXVIII), yellow needles (75 mg), m.p. 190°C.

Spectral data

$M^{+\cdot}$ m/e 328, analysed for $C_{18}H_{16}O_6$, NMR (100 MHz, $CDCl_3$) 2.30 (2H, dd, J=9, 2 Hz, ArH-2',6'), 3.05 (2H, dd, J=9, 2 Hz, ArH-3',5'), 3.48 (1H, s, ArH-8), 3.52 (1H, s, H-3), 4.00, 4.05 and 4.10 (3H each, s, 3x-OCH_3).

Acetylation of (LXVIII)

LXVIII (50 mg) was acetylated by heating it with acetic anhydride and pyridine (1 ml each) on a water bath for five hours, worked up by the addition of water and the precipitate filtered, washed with water and dried. Crystallisation from methanol gave colourless needles (LXIX, 40 mg), m.p. 137°C.

Spectral data

$M^{+\cdot}$ m/e 370, analysed for $C_{20}H_{18}O_7$; NMR (100 MHz, $CDCl_3$) 2.26 (2H, dd, J=9, 2 Hz, ArH-2',6'), 3.05 (2H, dd, J=9, 2 Hz-3',5'), 3.15 (1H, s, ArH-8), 3.76 (1H, s, H-3), 4.02, 4.10 and 4.14 (3H each, s, 3x-OCH_3).

Claisen rearrangement of (LXVII)

Compound (100 mg) in DMSO (5 ml) was refluxed under nitrogen atmosphere for five hours. The reaction mixture was poured into crushed ice and the solid obtained on filtration exhaustively washed with water so as to remove last traces of DMSO. Crystallisation from $CHCl_3$-petroleum ether gave the product (LXVI) as pale yellow needles (50 mg), m.p. 115°C.

Spectral data

$M^{+\cdot}$ m/e 369 analysed for $C_{19}H_{20}O_6$; NMR (60 MHz, $CDCl_3$), 2.20 (2H, dd, J=9, 2 Hz, ArH-2',6'), 2.97 (2H, dd, J=9, 2 Hz, ArH-3',5'), 4.05 (1H, m, $-CH_2-CH=CH_2$), 4.95 (2H, m, $-CH_2-CH=CH_2$), 5.95, 6.10 and 6.15 (3H each, s, $3x-OCH_3$), 6.40 (2H, d, $Ph-CH_2-CH=CH_2$).

Verbesina encelioides Benth

Isolation

Aerial parts of the air dried plant (4 Kg) were extracted thrice with petroleum ether in a percolator. The combined extract was taken to dryness under reduced pressure to yield a green oily mass (100 g) which was dissolved in petroleum ether (200 ml) and left in an ice chest for three days. The solid material which was deposited on the sides of the flask was filtered and was shown by TLC to be a mixture of two compounds. The solid mass (10 g) was dissolved in chloroform and absorbed on silica gel for column chromatography. Elution with petroleum ether and petroleum ether-benzene (1:1) gave only oily material which was not studied further. Elution with chloroform gave a solid, pseudo-taraxasteryl acetate (LXXIIb) whereas pseudo-taraxasterol (LXXIIa) came down with chloroform-methanol (95:5).

Pseudo-taraxasteryl acetate (LXXIIb)

It was purified by repeated crystallisations with chloroform-methanol, colourless needles (5 g), m.p. 208-209°C.

Spectral data

$M^{+\cdot}$ m/e 468, analysed for $C_{32}H_{52}O_2$; νmax (Nujol) 1725, 1245 cm^{-1}; λ max ($CHCl_3$) 210 nm. NMR (60 MHz, $CDCl_3$), 4.75 (1H, m, $-\underline{CH}=C\langle$), 5.40 (4/3H, t, $-\underline{CH}-O-\overset{O}{\overset{\|}{C}}-CH_3$ impurity), 7.95 (3H, s, $-O-\overset{O}{\overset{\|}{C}}-CH_3$), 8.40 (3H, brs, $\rangle C=CH_3$), 8.90-9.30 (7x-CH_3); M.S. 468 (16.2), 408 (13.1), 249 (15.6), 218 (23.1), 205 (14.1), 204 (33.1), 203 (24.1), 191 (24.9), 190 (35.1), 189 (100), 187 (11.0), 175 (20.3), 163 (10.3), 161 (17.7), 149 (16.2), 148 (10.4), 147 (20.2), 136 (30.0), 135 (39.2), 134 (19.5), 133 (23.9), 133 (27.9), 132 (26.1), 121 (49.9), 120 (15.4), 119 (27.3), 109 (47.1), 108 (23.9), 107 (39.4), 105 (19.5), 95 (55.3), 94 (15.5), 93 (32.7), 91 (13.7), 81 (37.6).

Attempted hydrogenation of pseudo-taraxasteryl acetate

Compound (200 mg) was dissolved in methanol (20 ml) and palladium charcoal (100 mg) added to it. Hydrogenation was discontinued after 10 minutes and the catalyst filtered off. Evaporation of the solvent and crystallisation of the residue from $CHCl_3$-petroleum ether gave needles (160 mg), m.p. 202-203°C. Its M.S. showed $M^{+\cdot}$ at 468 for $C_{32}H_{52}O_2$ and the NMR was identical with that of the starting material except that the intrusive signal at 4.70 was missing.

Osmylation of pseudo-taraxasteryl acetate

Compound (300 mg) was treated with OsO_4 (300 mg) in dry pyridine (10 ml) and the reaction mixture kept for three days at room temperature in the dark. Methanol (15 ml) and an aqueous solution of Na_2SO_3 (2 ml, 5%) were added to it and refluxed for three hours. The reaction mixture was worked up by addition of water and extraction with ether. The residue obtained on evaporation of ether was chromatographed over silica gel. Elution with chloroform-methanol (95:5) gave product which was crystallised from methanol to give colourless plates (LXXIII, 200 mg), m.p. 254-55°C.

Spectral data

$M^{+\cdot}$ m/e 502, analysed for $C_{32}H_{52}O_4$; NMR (60 MHz, $CDCl_3$) 5.50 (1H, t, $>\underline{CH}-O-\overset{O}{\overset{\|}{C}}-CH_3$), 6.35 (1H, brs, $>\underline{CH}-OH$), 7.98 (3H, s, $-O-\overset{O}{\overset{\|}{C}}-\underline{CH_3}$), 9.90-9.20 (9x$-CH_3$); M.S. 502 (2.2), 484 (2.5), 471 (13.5), 411 (16.9), 385 (10.7), 208 (13.6), 203 (16.8), 191 (44.5), 190 (22.9), 189 (70.3), 187 (13.4), 175 (21.5), 165 (10.1), 163 (16.8), 161 (16.7), 151 (11.9), 149 (18.4), 147 (18.9), 137 (20.7), 136 (40.8), 135 (46.5), 134 (14.0), 133 (20.6), 123 (20.8), 122 (18.0), 121 (44.3), 120 (11.4), 119 (26.9), 109 (48.1), 108 (12.5), 107 (46.9), 105 (18.2), 97 (13.0), 95 (69.8), 94 (13.5), 93 (35.2), 91 (12.9), 83 (21.0), 82 (14.5), 81 (84.8).

Reaction of pseudo-taraxasteryl acetate with SeO_2

Compound (500 mg) and SeO_2 (500 mg) were taken in dry dioxane (25 ml) and the mixture refluxed for 20 hours, worked up with the addition of cold water and extraction with ether. The brown gum obtained on evaporation of ether was purified by column chromatography over silica gel. Elution with chloroform-methanol (95:5) afforded the pure product which was crystallised from methanol to yield colourless plates (LXXIV, 100 mg), m.p. 239-40°C.

Spectral data

$M^{+\cdot}$ m/e 482, analysed for $C_{32}H_{50}O_3$; NMR (60 MHz, $CDCl_3$) 0.60 (1H, s, $=C-CH-O$), 3.30 (1H, m, $-C=CH-$), 5.50 (1H, m, $-CH-O-C(=O)-CH_3$), 7.98 (3H, s, $-O-C(=O)-CH_3$), 8.80-9.30 ($7\times-CH_3$).

Pseudo-taraxasterol (LXXIIa)

It was purified by repeated crystallisation with methanol, colourless needles (1.7 g), m.p. 196°C.

Spectral data

$M^{+\cdot}$ m/e 426, analysed for $C_{30}H_{50}O$; ν max (Nujol) 3300, 1040 cm^{-1}; λ max ($CHCl_3$) 210 nm; NMR (60 MHz, $CDCl_3$), 4.75 (1H, m, $>C=CH-$), 5.40 (1/2H, brs, impurity), 6.75 (1H, m, $>CH-OH$),

8.38 (3H, s, $\rangle C=CH_3$), 8.85-9.30 ($7\alpha-CH_3$). M.S. 426 (30), 408 (10), 218 (40), 207 (80), 203 (20), 190 (40), 189 (100), 135 (60), 123 (30), 122 (30), 121 (60), 119 (40), 109 (55), 108 (50), 95 (70), 91 (35).

Acetylation of pseudo-taraxasterol

Compound (100 mg) was kept overnight with acetic anhydride/ pyridine (2 ml each) at room temperature. The reaction mixture was worked up in the usual manner and the product crystallised from chloroform-methanol to yield colourless needles (90 mg), m.p. 205-206°C. It was found identical with the natural compound (Co-TLC, IR and NMR).

Reaction of pseudo-taraxasterol with DMSO-Ac_2O

A mixture of dimethyl sulfoxide and acetic anhydride (2 ml each) was heated on a water bath for about half an hour and compound (200 mg) added to it. The reaction mixture was kept for 24 hours at room temperature and then crushed ice was added to it. The solidified mass was filtered and washed exhaustively with cold water untill the last traces of dimethyl sulfoxide were removed. It was dried and crystallised from chloroform-petroleum ether to give colourless needles of (LXXV, 150 mg), m.p. 205-206°C.

Spectral data

$M^{+\cdot}$ m/e 496, analysed for $C_{32}H_{56}OS$; NMR (90 MHz, $CDCl_3$) 4.95 (1H, m, C=C<u>H</u>-), 5.44 (2H, s, -O-C<u>H</u>$_2$-S-), 5.50 (1/2H, brs, impurity), 6.95 (1H, m, $\rangle$C<u>H</u>-O-CH$_2$-S-), 7.90 (3H, s, -S-CH$_3$), 9.44 (3H, s, $\rangle$C=CH$_3$), 8.95-9.35 (7x-CH$_3$).

Excoecharia agallocha Linn

Isolation

Air dried heart wood of the plant (4 Kg) was exhaustively extracted with petroleum ether in a Soxhlet. TLC examination of the concentrated extract revealed the presence of two major components, both of which were revealed by spraying the plate with alcoholic solution of phosphomolybdic acid. The alkaloidal nature of the less polar of these was evident from the positive orange colour developed on spraying the plate with Dragendorff's reagent.

The extract was, therefore, chromatographed on a column of silica gel. Elution with petroleum ether and benzene gave mixtures of oily products which were not examined further. Elution with chloroform afforded the alkaloidal (LXXIX) and with chloroform-methanol (95:5) the non-alkaloidal (LXXVI) components.

(LXXIX)

The oil obtained from the column was highly contaminated with impurities having close R_f values. It was purified by repeated column chromatography to yield a light yellow oil (400 mg) which was found to be homogeneous on examination by TLC.

Spectral data

$M^{+\cdot}$ m/e 343, analysed for $C_{21}H_{29}NO_3$; ν_{max} (Nujol) 1650, 1600, 1498, 1425, 1290, 1250, 1215, 1100, 1015 and 780 cm^{-1}; λ_{max} ($MeOH$) 235, 285, 350 nm. NMR (60 MHz, $CDCl_3$), 2.38 (1H, d, J=16 Hz, Ph-CH=CH-C(=O)-), 2.80 (1H, d, J=9 Hz, ArH-6), 3.30 (1H, d, J=16 Hz, Ph-CH=CH-C(=O)-), 3.45 (1H, d, J=9 Hz, ArH-5), 4.96 (1H, m, $-CH_2-CH=C$), 6.28 and 6.38 (3H each s, 2x-OCH_3), 6.52 (4H, brs, $-N(CH_2-)_2$), 6.75 (2H, d, Ph-CH_2-CH=), 8.32 (3H, s, $=C(-CH_3)-$), 8.35-8.60 (9H, brs, $=C(-CH_3)-$ and $N(CH_2-CH_2)_2CH_2$). NMR (90 MHz, $CDCl_3$ + 8 drops of C_6D_6), 2.34 (1H, d, J=16 Hz, Ph-CH=CH-C(=O)-), 2.84 (1H, d, J=9 Hz, ArH-6), 3.32 (1H, d, J=16 Hz, Ph-CH=CH-C(=O)-), 3.62 (1H, d, J=9 Hz, ArH-5), 4.96 (1H, m, $-CH_2-CH=C$) 6.42 (6H, s, 2x-OCH_3), 6.50-6.85 (6H, m, $-N(CH_2-)_2$ and Ph-CH_2-CH=), 8.34 and 8.48 (3H each, s, 2x$=C(-CH_3)-$), 8.50-8.70 (6H, brs, $-N(CH_2-CH_2)_2CH_2$).

(LXXVI)

The $CHCl_3$ -MeOH (95:5) eluates were combined, concentrated and the solid obtained crystallised from methanol to give yellow shining plates (500 mg), m.p. 140°C. It was identified as 2',4',6',4-tetramethoxy chalcone by comparison with an authentic sample.

Spectral data

$M^{+\cdot}$ m/e 328, analysed for $C_{19}H_{20}O_6$; ν_{max} (Nujol), 1670, 1610, 1590 cm^{-1}; NMR (100 MHz, $CDCl_3$), 2.66 (2H, dd, J=9, 2 Hz, Ar<u>H</u>-2,6), 2.85 (1H, d, J=16 Hz, Ph-<u>CH</u>=CH-C(=O)-), 3.28 (2H, dd, J=9, 2 Hz, Ar<u>H</u>-3',5'), 3.38 (1H, d, J=16 Hz, Ph-CH=<u>CH</u>-C(=O)-), 3.98 (2H, s, Ar<u>H</u>-3',5'), 6.26 and 6.28 (3H each, s, 2x-OCH_3), 6.34 (6H, s, 2x-OCH_3).

Synthesis of (LXVI)

A solution of 2,4,6-trimethoxy acetophenone (2.10 g) and 4-methoxybenzaldehyde (1.36 g) in ethanol (95%, 20 ml) was treated with aqueous sodium hydroxide (2 g in 10 ml). The reaction mixture was kept at room temperature for 48 hours. It was then diluted with cold water, acidified with conc. HCl and extracted with ether. The ether extract was washed several times with water, dried over anhydrous Na_2SO_4 and evaporated to yield a solid which was purified by repeatedly crystallising it with methanol, light yellow prisms (2 g), it was found identical with the natural sample (IR, Co-TLC).

BIBLIOGRAPHY

BIBLIOGRAPHY

1. J.C. Roberts, Chem. Rev., (1961), 61, 591.

2. T.R. Seshadri and S. Neelakantam, Curr. Sci. (India), (1961), 30, 90.

3. 'Handbook of Naturally Occurring Compounds', Vol. 1, by T.K. Devon and A.I. Scott, (1975), Academic Press Inc., New York.

4. L.D. Antonaccio, L.G. Fonseca da Silva, D. de Barros Correa, O.R. Gottlieb and M.T. Magalhaes, Anais. Acad. Brasil. Ciene., (1965), 37 (2), 229; Chem. Abstr., (1966), 65, 2622.

5. O.R. Gottlieb, M.T. Magalhaes, M. Camey, A.A. Lins Mesquita and D. de Barros Correa, Tetrahedron, (1966), 22, 1777.

6. E.D. Burling, A. Jefferson and F. Scheinmann., Tetrahedron Letters, (1964), 599.

7. A. Jefferson and F. Scheinmann, Tetrahedron Letters, (1964), 1239; J. Chem. Soc.(C), (1966), 178.

8. J.A. Ballantine and C.T. Pillinger, Tetrahedron, (1967), 23, 1691.

9. A. Jefferson and F. Scheinmann, J. Chem. Soc. (C), (1966), 178.

10. E.A. Sherif, R.K. Gupta and M. Krishnamurty, Tetrahedron Letters, (1980), 641.

11. F.E. King, J.T. King and L.C. Manning, J. Chem. Soc.(C), (1957), 563.

12. F.M. Dean, B. Parton, A.W. Price, N. Sowrichien and D.A.H. Taylor, Tetrahedron Letters, (1967), 2737.

13. 'The Chemistry of Flavonoid Compounds', edited by T.A. Geissman, (1962), Pergamon Press, London, page 428.

14. H. Behme and T. Severin, Arch. Pharm., (1967), 300, 285.

15. H. Bohme and T. Severin, Arch. Pharm., (1957), 290, 448.

16. H. Bohme and T. Severin, Arch. Pharm., (1957), 290, 498.

17. L. Jurd and R.M. Horowitz, J. Org. Chem., (1957), 22, 1618.

18. 'The Flavonoids', edited by J.B. Harborne, T.J. Mabrya and Helga Mabry, (1975), Chapman and Hall, London, page 49.

19. O.R. Gottlieb, M.T. Magalhaes, M.O. da Silva Pareira, A.A. Lins Mesquita, D. de Barros Correa and G.G. de Oliviera, Tetrahedron, (1960), 24, 1601.

20. O.R. Gottlieb, A.A. Lins Mesquita, G.G. de Oliveira and M. Teixeira de Melo, Phytochemistry, (1970), 9, 2537.

21. N.D. Locksley and I.G. Murray, Phytochemistry, (1971), 10, 3179.

22. S. Ghosal, S. Banerjee, R.B.S. Chauhan and R.S. Srivastava, J. Chem. Soc. (Perkin I), (1977), 7, 741.

23. N.D. Locksley, I. Moore and F. Scheinmann, J. Chem. Soc.(C), (1966), 2186.

24. O.R. Gottlieb, M.T. Magalhaes and G.M. Stefani, Tetrahedron, (1966), 22, 1735.

25. D.L. Dreyer, Dies. Abs., (1960), 21, 1374.

26. M.L. Wolfrom, P. Komitsky and J.H. Looker, J. Chem. Soc.(C), (1955), 3992.

27. B. Jackson, N.D. Locksley, I. Moore and F. Scheinmann, J. Chem. Soc.(C), (1969), 2579.

28. B. Jackson, N.D. Locksley and F. Scheinmann, J. Chem. Soc.(C), (1966), 178.

29. G.D. Shah and R.C. Shah, J. Sci. Ind. Res. (India), (1958), 18B, 630.

30. P. Yates and G.N. Stout, J. Am. Chem. Soc., (1958), 80, 1691.

31. S.P. Gunasekara, K. Sivapalan, M.U.S. Sultanbawa and W.D. Ollis, J. Chem. Soc. (Perkin I), (1977), 11.

32. H.D. Locksley and F. Scheinmann, J. Chem. Soc.(C), (1966), 430.

33. 'Advances in the Spectroscopy of Plant Phenolics' by V.M. Chari and H. Wagner, in 'Recent Advances in Phytochemistry', Vol.12, edited by T. Swain, J.B. Harborne and C.F. Van Sumere, (1979), Plenum Press, New York.

34. E. Ritchie and W.C. Taylor, Tetrahedron Letters, (1964), 1431.

35. G. Kartha, H.N. Ramchandran, A.B. Bhat, P.M. Nair, V.K. Raghvan and K. Venkataraman, Tetrahedron Letters, (1963), 459.

36. W.D. Ollis, M.V.J. Ramsay, I.O. Sutherland and S. Mongkolsuk, Tetrahedron, (1965), 21, 1453.

37. M.L. Wolfrom, P. Komitsky(Jr.) and J.H. Looker, J. Org. Chem., (1965), 30, 144.

38. A. Arnone, G. Cardillo, L. Merlini and R. Mondelli, Tetrahedron Letters, (1967), 4201.

39. V.K. Bhatia, J.D. Ramnathan and T.R. Seshadri, Tetrahedron, (1967), 23, 1363; Tetrahedron Letters, (1969), 907.

40. K. Hostettmann and H. Wagner, Phytochemistry, (1977), 16, 821.

41. M. Kaldas, I. Miura and K. Hostettmann, Phytochemistry, (1979), 17, 295.

42. A.J. Quillinan and F. Scheinmann, J. Chem. Soc. (Chem. Comm.), (1971), 966.

43. K.A. Bell, I.J. Flatman, P. Golborn, A. Pachl and F. Scheinmann, J. Chem. Soc. (Chem. Comm.), (1976), 900.

44. H.G. Floss and A. Rettig, Z. Naturforsch., (1964), 19b, 1103.

45. P. Gupta and J.R. Lewis, J. Chem. Soc.(C), (1971), 629.

46. D.H.R. Barton and T. Cohen, Festschr A. Stoll; Birkhauser, Basle (1957), page 117.

47. D.H.R. Barton and A.I. Scott, J. Chem. Soc.(C), (1958), 5074.

48. J.R. Lewis and B.H. Warrington, J. Chem. Soc.(C), (1964),5074.

49. J.E. Atkinson and J.R. Lewis, J. Chem. Soc.(C), (1969), 261.

50. J.W.A. Findlay, P. Gupta and J.R. Lewis, J. Chem. Soc.(C), (1969), 2761.

51. Masé Fujita and Takao Inoue, Chem. Pharm. Bull., (1960), 28, 3478.

52. A.G. McInnes, D.G. Smith, J.A. Walter, L.C. Vining and J.L.C. Wright, J. Chem. Soc. (Chem. Comm.), (1975), 66.

53. J.S.E. Holker, R.D. Lapper and J. Simpson, J. Chem. Soc. (Perkin I), (1974), 2135.

54. A. Kamal, S.A. Hussain and A.A. Qureshi, Pakistan J. Sci. Ind. Res., (1971), 90 and 104.

55. K.K. Chexal, C. Fouweather, J.S.E. Holker, T.J. Simpson and K. Young, J. Chem. Soc. (Perkin I), (1974), 1584.

56. A.J. Birch, J. Baldas, J.R. Hlubucek, T.J. Simpson and P.W. Westerman, J. Chem. Soc. (Perkin I), (1976), 893.

57. 'Isoprene Units in Natural Phenolic Compounds' by W.D. Ollis and I.O. Sutherland, in 'Recent Development in the Chemistry of Natural Phenolic Compounds', edited by W.D. Ollis, Pergamon Press, (1961), page 84.

58. I.M. Campbell, C.M. Calzadilla and N.J. McCorkindale, Tetrahedron Letters, (1966), 5107.

59. G. Cardillo, R. Criechio and L. Merlini, Tetrahedron Letters, (1968), 4825; ibid, (1969), 907.

60. H.D. Locksley, A.J. Quillinan and F. Scheinmann, J. Chem. Soc.(C), (1971), 3804.

61. I. Carpenter, R.J. McGarry and F. Scheinmann, Tetrahedron Letters, (1969), 3783.

62. A.C. Jain, P. Lal and T.R. Seshadri, Tetrahedron, (1960), 28, 1977.

63. T. Meikle and R. Stevens, Tetrahedron Letters, (1972), 4787.

64. V.M. Deshpande, A.V. Rao, S.R. Rama and K. Venkataraman, Ind. J. Chem., (1972), 10, 661.

65. R. Ameja, S.K. Mukherjee and T.R. Seshadri, Tetrahedron, (1958), 4, 286.

66. A.J. Birch, Chemical Society (London), Special Publication, (1958), 12, 4.

67. R.N. Chopra, S.L. Nayer and I.C. Chopra, 'Glossary of Indian Medicinal Plants', (1956), C.S.I.R., New Delhi, page 46.

68. 'Wealth of India', Council of Scientific and Industrial Research, Delhi, (1950), Vol. II, page. 17.

69. T.R. Govindachari, B.R. Pai, N. Muthukumaraswamy, V.R. Rao and N. Mityanand Rao, Ind. J. Chem., (1968), 6, 87.

70. G.H. Stout, M.M. Krahn and G.D. Brook, Tetrahedron Letters, (1968), 3285.

71. A. Shoeb, M.D. Manchanda, R.S. Kapil and S.P. Popli, J. Chem. Soc. (Chem. Comm.), (1975), 281.

72. 'The Systematic Identification of Flavonoid Compounds' by T.J. Mabry, K.R. Markham and M.B. Thomas, (1970), Springer-Verlag, Berlin.

73. Y.N. Sharma, A. Zaman and A.R. Kidwai, Tetrahedron, (1966), 22, 3221.

74. F.D. Cramer, Rev. Pure and Appl. Chem., (1955), 5, 143.

75. S.G. Frank, J. Pharm. Sci., (1975), 64, 1585.

76. H.M. Powell, 'Non-Stoichiometric Compounds', (1964), 488.

77. C. Asselineau and J. Asselineau, Ann. Chim. (Paris), (1964), 9, 461.

78. S. Karunanayake, S. Sotheeswaran and M.U.S. Sultanbawa, Tetrahedron Letters, (1979), 1449.

79. G.G. Stout, R.A. Alden, J. Kraut and D.F. High, J. Am. Chem. Soc., (1962), 2053.

80. L.A. Paquette and H. Stucki, J. Org. Chem., (1966), 31, 1231.

81. R. Grigg, J.A. Knight and P. Roffey, Tetrahedron, (1966), 22, 3301; R.G. Wilson, J.H. Bowie and D.H. Williams, Ibid, (1967), 24, 1407; H.D. Locksley, Zechmeister's Progress in the Chemistry of Organic Natural Products, edited by W. Herz, H. Grisebach and G.W. Kiby, (1973), Springer-Verlag.

82. G.G. Karanjgaokar, A.V. Rama Rao, K. Venkataraman and S.S. Yamul, Tetrahedron Letters, (1973), 4977.

83. M. Dahanayake, I. Kitigawa, R. Somanathan and M.U.S. Sultanbawa, J. Chem. Soc. (Perkin I), (1974), 2513.

84. E. Ritchie, W.C. Taylor and J.S. Shannon, Tetrahedron Letters, (1964), 1437.

85. I. Carpenter, H.D. Locksley and F. Scheinmann, Phytochemistry, (1969), 8, 2013.

86. F. Scheinmann and M.A. Stripong, Phytochemistry, (1971), 10, 1331.

87. Faik Shalan Al Jeboury and H.D. Locksley, Phytochemistry, (1971), 10, 603.

88. M. Minhal, H. Khan, S.K. Kapoor and Asif Zaman, Tetrahedron, (1976), 32, 749.

89. M. Shabbir, Asif Zaman, L. Crombie, S. Tuck and D.A. Whiting, J. Chem. Soc.(C), (1968), 1899.

90. M. Shabbir and Asif Zaman, Tetrahedron, (1970), 26, 5041.

91. R.N. Chopra, S.L. Nayer and I.C. Chopra, 'Glossary of Indian Medicinal Plants, (1956), C.S.I.R., New Delhi, page 244-45.

92. A.R. Kidwai, K.C. Saloojn, V.N. Sharma and Salimuzzaman Siddiqi, J. Sci. Ind. Res. (India), (1949), 8B, 115.

93. A. Khaleque, M.A. Wahed Miah, M. Sayeedul Huq and Kh. Abul Bashar, Sci. Res. (Pak.), (1970), VII, 61.

94. D.G.I. Kingston, Tetrahedron, (1971), 27, 2691.

95. H. Budzikiewicz, C. Djerassi and D.H. Williams, 'Mass Spectroscopy of Organic Compounds', Holden-Day (1967), p. 168-69.

96. R. Chong and P.S. Clezy, Tetrahedron Letters, (1966), 7, 741.

97. H. Khan and Asif Zaman, Tetrahedron, (1974), 30, 2811.

98. R.K. Gupta and M. Krishnamurti, Phytochemistry, (1977), 16, 1104.

99. H. Wagner, O. Seligmann, L. Horhammer, M. Negradi, L. Farkas, J. Strelisky and V.S. Verma, Acta. Chim. Acad. Sci. Hung., (1968), 57, 169.

100. J. Shinoda, J. Pharm. Soc. Japan, (1928), 48, 314.

101. R.D.H. Murray and M.M. Ballantyne, Tetrahedron, (1970), 26, 4667.

102. See ref. 48.

103. R.C. Sharma, A. Zaman and A.R. Kidwai, Phytochemistry, (1968), I, 1091.

104. S.R. Gupta, T.R. Seshadri, C.S. Sharma and N.D. Sharma, Ind. J. Chem., (1975), 13, 785.

105. R.J. Highet and P.J. Highet, J. Org. Chem. (1965), 30, 902.

106. L. Canonica, P. Grametica, P. Manitto and D. Monti, J. Chem. Soc. (Chem. Comm.), (1979), 1073.

107. O.R. Gottlieb, Jose, G.S. Maia and Jamil C. Mourao, Phytochemistry, (1976), 15, 1289.

108. F. Bohlmann et al., Phytochemistry, (1978), 17, 453.

109. H.P. Tewari and V.S. Rao, Ind. J. Chem., (1975), 16B, 1133.

110. C.W. Glemnie and S.C. Jain, Phytochemistry, (1990), 19, 157.

111. K.V. Gopinath, Lalit Prakash and A.R. Kidwai, J. Sci. Ind. Res. (India), (1962), 21B, 404.

112. H. Budzikiewies, J.M. Wilson and Carl Djerassi, J. Am. Chem. Soc., (1963), 85, 3698.

113. 'Advances in Organic Chemistry, Methods and Results', Vol. 6, edited by Edward C. Taylor and Hans Wynberg, (1969), Interscience Publishers, p. 349-50.

114. 'Wealth of India', Council of Scientific and Industrial Research, Delhi, (1950), Vol. III, page 234-35.

115. O.P. Gupta, S.C. Gupta, K.L. Dhar and C.K. Atal, Phytochemistry, (1978), 17, 601.

116. S.M. Spatz and Kersl, J. Org. Chem., (1959), 24, 1381.

Wightianone–Palmitic Acid, a Clathrate from *Calophyllum wightianum*

By Francis M. Dean
(*Robert Robinson Laboratories, University of Liverpool, Liverpool* L69 3BX)
Hafizzullah Khan, Najme Minhaj, and Satya Prakash
(*Department of Unani Medicine, Aligarh Muslim Uni ersity, Aligarh*-202001, *India*)
and Asif Zaman
(*Postgraduate Department of Chemistry, University of Kashmir, Srinagar*-190006, *India*)

Reprinted from the Journal of The Chemical Society
Chemical Communications 1980

Wightianone–Palmitic Acid, a Clathrate from *Calophyllum wightianum*

By FRANCIS M. DEAN
(*Robert Robinson Laboratories, University of Liverpool, Liverpool* L69 3BX)
HAFIZZULLAH KHAN, NAJME MINHAJ, and SATYA PRAKASH
(*Department of Unani Medicine, Aligarh Muslim Uni ersity, Aligarh*-202001, *India*)
and ASIF ZAMAN
(*Postgraduate Department of Chemistry, University of Kashmir, Srinagar*-190006, *India*)

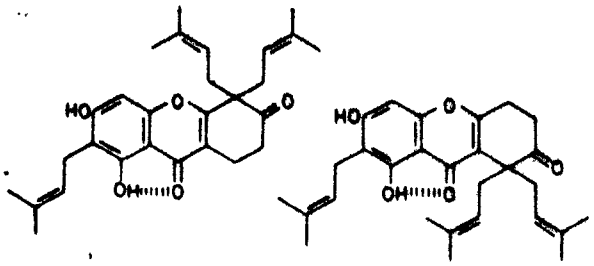
Summary The heartwood of *Calophyllum wightianum* T. Anders gives, upon extraction with benzene, a clathrate composed of 4 moles of wightianone (**1**), a new member of the rare tetrahydroxanthone series of phenols, and 1 mole of fatty acid, mainly palmitic.

FROM the heartwood of *Calophyllum wightianum* T. Anders (Guttiferae) benzene extracts a yellow phenol, wightianone, which seemed to fit none of the common categories of heartwood phenols. Moreover, the ^{1}H n.m.r. spectrum required the presence of 40 protons whereas accurate mass measurements indicated a molecular ion *m/e* 450·2493 incompatible with any molecular formula in the series $C_xH_{4n}O_y$. After many attempts at further purification by chromatography and crystallisation we noted that a six-proton singlet at δ 1·25 varied slightly in relative intensity. This band was therefore assumed to be intrusive in its entirety and the pigment was assigned the molecular formula $C_{30}H_{34}O_5$ and eventually the structure (**1**). Because material was very limited the only reaction studied in detail was hydrogenation, which furnished a hexahydro-derivative assigned the molecular formula $C_{30}H_{46}O_5$ from n.m.r. and mass spectral results on the assumption that intrusive protons were no longer present.

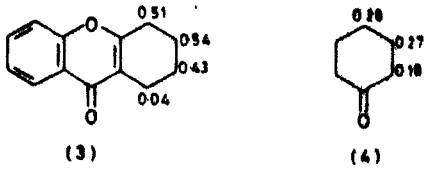
The usual evidence readily established the presence of one chelated and one rather acidic phenolic proton along with one carbonyl group (ν_{max} 1700 cm^{-1}; ^{13}C n.m.r. δ 212 p.p.m. in the hexahydro-derivative), as in a saturated acyclic ketone or a cyclohexanone system, and another carbonyl group in a highly conjugated and/or hydrogen bonded situation (ν_{max} 1645 cm^{-1}; ^{13}C n.m.r. δ 181·4 p.p.m. in the hexahydro-derivative). These results pointed to a 5,7-dihydroxy-chromone nucleus and this was confirmed by the u.v. spectrum (λ_{max} 230, 258, 300, and 330 nm) and general correspondence with chromones related to peucenin.[1,2]

Three prenyl substituents were disclosed by mass spectroscopic losses of C_3H_5, C_4H_6, and C_5H_8 fragments from the molecular ion to give the strongest peaks in the spectrum,[3] the ^{1}H n.m.r. spectrum showed the requisite bands for

δ vinylic protons and 6 vinylic methyl groups together with bands for 3 methylene groups only one of which was attached to aryl carbon (benzylic methylene resonance at δ 3·48). This leaves one vacant aromatic position between two oxygen atoms (ArH, δ 6·34) but does not indicate which one. A strong positive Gibbs test[3,4] requires a free position *para* to the hydroxy group and so orientates the phenolic system in (1).



(1) (2)



(3) (4)

The other two prenyl groups are attached to sp^3 carbon but the methylene resonances, though at two different fields (δ 3·16 and 2·70), cannot indicate two different methylene groups. The splitting patterns and double irradiation experiments show that each 2-proton band consists of one resonance from each methylene group *i.e.* there are two *identical* spin systems $-CH_AH_BCH_X{=}C<$ (J_{AB} 7·7, J_{BX} 8·5, and J_{AX} 13·5 Hz) superimposed and therefore two prenyl groups identically situated.

The ^1H n.m.r. evidence (in $CDCl_3$) finally shows that the remaining protons belong to the grouping CH_2CH_2 attached at each end to sp^3 carbon. This leaves only one carbon atom unassigned, and the two equivalent prenyl groups must be attached to it, the splitting pattern being a consequence of diastereotopicity. Assembly of these structural features leads only to structures (1) and (2). Because material was very limited, we sought to distinguish between these structures by the aromatic solvent shift method using benzene,[5] which caused the ring methylene resonances to move upfield and almost to the same position so that the original two triplets became a somewhat broadened singlet. Relevant shifts for tetrahydroxanthone[6] (3) and cyclohexanone (4) are shown in the diagrams; assuming solvent shifts to be approximately additive, we now expect solvent shifts for structure (1) of 0·61 and 0·31 p.p.m. and for structure (2) of 0·72 and 0·78 p.p.m. The pigment actually suffers shifts of 0·59 and 0·29 p.p.m. and therefore has structure (1).

The intrusive 6-proton singlet seemed likely to originate from a long chain of methylene groups and there is, just clear of the noise, a triplet at δ 0·88 that could be ascribed to a terminal methyl group while a similar triplet at δ 2·37 suggested methylene attached to sp^2 carbon and so the presence of a fatty acid. A rough determination of the methyl–methylene ratio gave for the acid a size between dodecanoic and stearic acids. Since conventional means had failed to separate the pigment from the acid, the pigment was dissolved in warm methanol saturated with urea so that, when this crystallised first, it would tend to take the acid with it as a clathrate.[7] Two successive treatments reduced the intrusive band to a relative intensity of about 1 proton but losses were too great for the separation to be completed.

The acid recovered from the urea crystallizate was found by methylation (CH_2N_2) and g.l.c. analysis to be palmitic with a little myristic. The original pigment had had m.p. 167 °C, whereas the 'acid-free' pigment melted at *ca.* 130 °C, a fall of *ca.* 35 °C showing that the original pigment was itself an inclusion compound. This was then reconstituted by crystallising the 'pure' pigment with authentic palmitic acid and had m.p. 160 °C and was otherwise indistinguishable from the original material except that the intrusive band was somewhat stronger (clathrates are seldom perfectly stoicheiometric). Relative proton intensities showed the pigment and acid to be in the molar ratio 4 : 1, as are many similar inclusion compounds between deoxycholic acid and fatty acids.[7] It is also interesting that this one crystallises in long, regular hexagonal prisms as do the urea clathrates.

A pigment named zeyloxanthonone and assigned structure (2) has been reported in a related plant, *Calophyllum zeylanicum*.[8] No evidence was advanced for the orientation of the aromatic part and clathrate formation has not been discussed. It is therefore possible that the two pigments are identical.

(*Received*, 16th *January* 1980; *Com.* 047.)

[1] A. Bolleter, K. Eiter, and H. Schmid, *Helv. Chim. Acta*, 1951, **34**, 190.
[2] F. M. Dean, B. Parton, A. W. Price, N. Somvichitn, and D. A. H. Taylor, *Tetrahedron Letters*, 1967, 2737.
[3] M. Dahanayake, I. Kitagawa, R. Somanathan, and M. U. S. Sultanbawa, *J.C.S. Perkin I*, 1974, 2510, E. Ritchie, W. C. Taylor, and J. S. Shannon, *Tetrahedron Letters*, 1964, 1487.
[4] F. E. King, J. T. King, and L. C. Manning, *J. Chem. Soc.*, 1957, 563.
[5] P. Laslow, *Progr. N.M.R. Spectroscopy*, 1967, **3**, 231.
[6] L. A. Paquette and H. Stucki, *J. Org. Chem.*, 1966, **31**, 1238.
[7] F. D. Cramer, *Rev. Pure Appl. Chem.*, 1955, **5**, 143; S. G. Frank, *J. Pharm. Sci.*, 1975, **64**, 1585, H. M. Powell in 'Non-Stoicheiometric Compounds', ed. L. Mandelcorn, Academic Press, New York, 1964, p. 488; C. Assilineau and J. Asselineau, *Ann. Chim.* (*France*), 1964, **9**, 491.
[8] S. Karunanayake, S. Sotheeswaran, and M. U. S. Sultanbawa, *Tetrahedron Letters*, 1979, 1449.